NITROGEN CYCLING IN AN INTEGRATED
BIOMASS FOR ENERGY" SYSTEM

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1986

ACKNOWLEDGMENTS

[illegible] reports results from a project [illegible] program between the Institute of Food a[illegible] [illegible] the University of Florida and the Gas R[illegible] (GRI), entitled "Methane from Biomass and Waste[illegible] [illegible] from the IFAS/GRI cooperative program is gratefully acknowledged.

[illegible] P. A. Graetz was chairman and Dr. K. R. Reddy was co[illegible] advisory committee. Their friendship and guidance [illegible] [illegible]. The other members of the committee were Dr. [illegible] [illegible] A. Fiskell, and Dr. R. A. Nordstedt.

[illegible] thanks go to Bill Pottier who ran numerous [illegible] [illegible]ch. Other people who provided assistance [illegible] [illegible] Bill Christy, Stephen McCracken, Peter Krottje, [illegible] Campbell, Premila Rao, Ed Hopwood and Dave [illegible] [illegible] designed the statistical analyses. I appreciate [illegible] [illegible] facilities for fiber analyses. Carolyn [illegible] [illegible] typed the majority of this dissertation [illegible]

[illegible], this package is dedicated to my parents, sisters [illegible] [illegible] always gave me a boost to carry on.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

NITROGEN CYCLING IN AN INTEGRATED "BIOMASS FOR ENERGY" SYSTEM

By

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May, 1986

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A series of experiments were conducted to evaluate N cycling in three components of an integrated "biomass for energy" system, i.e. water hyacinth production, anaerobic digestion of hyacinth biomass, and recycling of digester effluent and sludge. Plants assimilated 50 to 90% of added N in hyacinth production systems. Up to 28% of the total plant N was contained in hyacinth detritus. Nitrogen loading as plant detritus into hyacinth ponds was 92 to 148 kg N ha^{-1} yr^{-1}

Net mineralization of plant organic ^{15}N during anaerobic digestion was 35 and 70% for water hyacinth plants with low (10 g N kg^{-1} dry tissue) and high (35 g N kg^{-1}) N content, respectively. Approximately 20% of the ^{15}N was recovered in the digested sludge while the remaining ^{15}N was recovered in the effluent.

Water hyacinth growth in digester effluents was affected by electrical conductivity (0.7 to 6.7 dS m^{-1}) and $^{15}NH_4^+$-N concentration

(23 to 289 mg N L^{-1}). Biomass yields were maximum at electrical conductivities of < 2.5 dS m^{-1} and $^{15}NH_4^+$-N concentrations of < 100 mg N L^{-1}.

Addition of water hyacinth biomass to soil resulted in decomposition of 39 to 50% of added C for fresh plant biomass and 19 to 23% of added C for digested biomass sludge. Only 8% of added ^{15}N in digested sludges was mineralized to $^{15}NO_3^-$-N despite differences in initial N content (27 and 38 g N kg^{-1} dry sludge). In contrast, 3 and 33% of added ^{15}N in fresh biomass with low and high N content, respectively, was recovered as $^{15}NO_3^-$-N.

Total ^{15}N recovery after anaerobic digestion ranged from 70 to 100% of the initial plant biomass ^{15}N. Land application of digester sludge resulted in the mineralization of 2% of initial biomass ^{15}N into plant available form. Use of water hyacinth for digester effluent treatment resulted in recycling of 21 to 38% of the initial biomass ^{15}N. Total N recovery by sludge and effluent recycling in the integrated "biomass for energy" system was 48 to 80% of the initial plant biomass ^{15}N. The remaining ^{15}N was lost from the system during anaerobic digestion and effluent recycling.

INTRODUCTION

Several types of aquatic plants are widely distributed in freshwater lakes and streams. These plants assimilate nutrients and produce biomass, which could potentially be used for beneficial purposes. Water hyacinth (*Eichhornia crassipes* [Mart] Solms) is one of the dominant aquatic plants distributed throughout the tropical and subtropical regions of the world. This freshwater macrophyte has already been evaluated for use in treating nutrient-enriched waters such as sewage effluent (Cornwell et al., 1977; Wolverton and McDonald, 1979; Reddy et al., 1985), agricultural drainage water (Reddy and Bagnall, 1981, Reddy et al., 1982), anaerobic digester effluent (Hanisak et al., 1980), and fertilized fish ponds (Boyd, 1976). The characteristics that make this plant grow rapidly in polluted waters make it an ideal candidate for large-scale nutrient removal and water purification (Reddy and Sutton, 1984).

An integrated aquaculture system has been developed using water hyacinth for water treatment and for total resource recovery. The components of an integrated aquaculture system are schematically illustrated in Fig. 1. Water hyacinth plants have been used for wastewater treatment while the biomass produced was harvested periodically and processed through anaerobic digestion to produce methane. The process produced a waste by-product which must be disposed of, or preferably utilized, in an environmentally-safe manner.

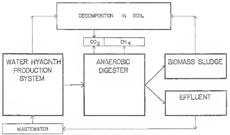

Figure 1 Integrated water hyacinth aquaculture system of biomass production, bioconversion to methane and digester waste recycling

The waste by-product contains digested biomass sludge (solid) and effluent (liquid). The digested biomass sludge was applied to soil as a nutrient source for plants. The effluent was recycled in water hyacinth ponds for nutrient recovery by plants. This type of integrated system will provide low cost water treatment and total resource recovery. Efficient utilization of by-products could potentially reduce the cost effectiveness of the system.

The overall objective of this study was to assess nitrogen cycling in the three components of an integrated "biomass for energy" system. Nitrogen is often identified as a limiting factor for plant growth and is used to establish loading rates in the disposal of solid and liquid waste. Information on N cycling is limited to studies on the individual components of the integrated system, i.e. the water hyacinth production system (Boyd, 1976, DeBusk et al., 1983, Reddy, 1983), anaerobic digestion (Hashimoto et al., 1980; Field et al., 1984), and effluent and sludge recycling (Ryan et al, 1973, Hanlsak et al., 1980; Terry et al., 1981; Atalay and Blanchar, 1984). No attempt has been made to establish N cycling within the entire integrated system.

The specific objectives of this study were 1) to determine growth rate and detritus production of water hyacinth grown in eutrophic lake water; 2) to determine the effect of detritus on N transformations in water hyacinth systems; 3) to evaluate N and C mineralization during anaerobic digestion of water hyacinth biomass, 4) to evaluate the potential of water hyacinth to grow in anaerobic digester effluents for N recovery, and 5) to determine N and C mineralization during decomposition of fresh and digested biomass added to soil

LITERATURE REVIEW

The three components of the integrated "biomass for energy" system were 1) the water hyacinth production system; 2) anaerobic digestion of water hyacinth biomass; and 3) recycling of digested biomass sludge and effluent. An integrated approach of wastewater renovation using aquatic macrophytes with utilization of biomass for energy production is economically appealing.

Water Hyacinth Biomass Production

The first component of the integrated "biomass for energy" system was an aquatic system for the production of biomass as well as water quality improvement. Although several aquatic plants naturally grow in polluted waters, one the most productive plants appears to be water hyacinth (Reddy et al., 1983).

Water hyacinth is a mat-forming, free-floating vascular aquatic plant with wide distribution in sub-tropical and tropical regions. The plant consists of a submerged rooting system and an aerial photosynthetic petiole and leaf (shoot) system (Fig. 2). The roots and aerial shoots are produced at the numerous nodes of the vegetative portion of a typically submerged rhizome (Penfound and Earle, 1948). The aerial buds, from which flowers and fruit clusters develop, are produced from the reproductive portion of the rhizome. Occasionally, the internodes of the rhizome expand and form new offsets.

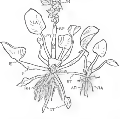

Figure 2. A generalized diagram of a water hyacinth plant. The major morphological structures are adventitious roots (AR), root hairs (RA), rhizome (RH), stolon (ST), detritus tissue (DT) attached to the plant, float (F), leaf isthmus (IS), leaf petiole (PT), peduncle (PD); spathe (SP); leaf lamina (LA), inflorescence (IN).

The elongated internodes were designated as stolons (Penfound and Earle, 1948)

The relatively rapid rate of colonization by water hyacinth is due primarily to vegetative reproduction (stolon and offset production) The plants reproduce sexually during warmer months until freezing terminates anthesis The developing fruits containing the seeds are cast off onto the mat or into the water They sink in water and remain in a viable condition for several years. Manson and Manson (1958) reported that each plant could produce 5000 to 6000 seeds which remained viable for at least 5 years.

The geographical distribution of water hyacinth is regulated by temperature and salt concentration in the water When average minimum temperature reached 10°C, productivity of water hyacinth approached zero (Reddy and Bagnall, 1981) Optimum growth was found in a temperature range of 25 to 30°C (Bock, 1969, Knipling et al , 1970). Water hyacinth is basically a freshwater plant and will die in waters with sustained salt concentrations in excess of 2500 mg L^{-1} (Haller et al., 1974)

Water hyacinth growth is regulated by the nutrient composition of the water medium, temperature, solar radiation, and plant density Water hyacinth potentially could be grown in nutrient enriched waters such as sewage effluents, agricultural runoff and drainage effluents, methane digester effluents, and runoff from animal waste operations Nitrogen is present as NH_4^+-N, NO_3^--N, and organic N in water and is available for water hyacinth production Organic N often predominates in most water media and is not readily available for plant assimilation. Water hyacinths are efficient users of inorganic N and plant assimilation is one of the major processes of N removal in hyacinth ponds

Water hyacinth adapted to light intensity and full sunlight elicited the greatest photosynthetic rate (Patterson and Duke, 1979). Optimum plant density to obtain maximum biomass yield varied with season and available plant nutrients in the water (Reddy and Sutton, 1984). DeBusk et al. (1981) and Reddy et al. (1983) established that optimum plant density for achieving maximum growth cultured in wastewaters was in the range of 15 to 35 kg wet wt m^{-2}.

Water hyacinth productivity has been evaluated in natural and nutrient-enriched waters. Growth rates of 2 to 29 g dry wt m^{-2} day^{-1} were reported for plants growing in natural waters of central and south Florida (Yount and Crossman, 1970; DeBusk et al., 1981). A wide-range of productivity (5 to 42 g dry wt m^{-2} day^{-1}) was recorded for plants cultured in nutrient-enriched waters (Schwegler and Kim, 1981, Hanisak et al., 1980). Reddy and DeBusk (1984) obtained an average of 52 and a maximum of 64 g dry wt m^{-2} day^{-1} for water hyacinths grown in nutrient-nonlimiting conditions.

The effectiveness of water hyacinth in removing inorganic N was reported for several nutrient-enriched wastewaters. Sheffield (1967) and Clock (1968) reported a 75 to 94% reduction of inorganic N from secondary sewage effluent in systems containing water hyacinths. Reddy et al. (1982) observed a 78 to 81% reduction of inorganic N from organic soil drainage water containing water hyacinths. Hanisak et al. (1980) concluded that 65% of N in digester effluents could be assimilated when water hyacinths were grown in diluted effluents. Boyd (1976) calculated average rates of N and P removal were 3.4 and 0.43 kg ha^{-1} day^{-1} in fertilized fish ponds. Rogers and Davis (1972) concluded that water hyacinth removal capacities were less effective with increasing nutrient concentrations.

The potential productivity and nutrient removal capacities of water hyacinth has led to its selection as a biomass feedstock for methane generation while providing a means for treatment of nutrient-enriched waters Extensive research, both in laboratory and field applications, was conducted on the use of water hyacinth in wastewater treatment during the past 20 years (Sheffield, 1967, Boyd, 1970a, Steward, 1970, Scarsbrook and Davis, 1970, Rogers and Davis, 1971, Dunigan et al , 1975, Cornwell et al , 1977; McDonald and Wolverton, 1980, Reddy et al , 1982; DeBusk et al., 1983) Water hyacinth was shown to be effective in removing N, P and other nutrients, and reducing biological oxygen demand and total suspended solids Water hyacinth was also shown to readily absorb and concentrate heavy metals (Wolverton and McDonald, 1975a,b, Cooley et al , 1978)

Nitrogen Cycling in the Water Hyacinth Production System

Nitrogen transformations occurring in a water hyacinth production system include 1) plant uptake, 2) mineralization/immobilization, 3) nitrification; 4) denitrification, and 5) NH_3-N volatilization (Fig 3)

Plant uptake is one of the major processes for N removal from water hyacinth-based wastewater systems Plant uptake is directly related to the growth rate and the nutrient composition of the water. Water hyacinth was more efficient in utilizing NH_4^+-N than NO_3^--N when both forms were supplied in equal proportions (Reddy and Tucker, 1983)

A dense cover of floating water hyacinths will regulate dissolved O_2, temperature and pH of water which influences several N transformations Generally, diel fluctuations of these water parameters were reported to be lower in areas covered with water hyacinths compared to open areas (Rai and Munshi, 1979, McDonald and Wolverton, 1980; Reddy, 1981)

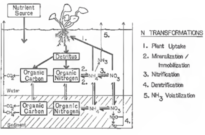

Figure 3. Nitrogen cycling in a water hyacinth production system. Nitrogen transformations investigated during this study are indicated with larger arrows.

High O_2 consumption within a water hyacinth mat during microbial decomposition of plant detritus (dead and decaying plant debris) could create anaerobic conditions (Boyd, 1970, McDonald and Wolverton, 1980,). The bulk of detritus was trapped within the mat and decomposed primarily at the water surface (DeBusk et al., 1983). Rate of inorganic N release from decomposing detritus depended on dissolved O_2 concentration of the water, C/N ratio, and temperature (Ogwada, 1983).

Low O_2 concentrations create conditions less favorable for nitrification and promote denitrification which may proceed within the water hyacinth mat, in the water column, or in the underlying sediment. Denitrification occurred primarily in the underlying sediment and the rate depended on diffusion of NO_3^--N from the water column to the sediment (Engler and Patrick, 1974; Reddy and Graetz, 1981).

Water temperatures were lower in areas covered with plants compared to open areas (Rai and Munshi, 1979, McDonald and Wolverton, 1980; Schreiner, 1980). A dense mat over the water surface served as a blanket barrier for exchange of heat between the atmosphere and the water (Rai and Munshi, 1979). Water hyacinths growing in either acid or alkaline water had a tendency to alter the pH towards neutrality (Haller and Sutton, 1973). A pH of 7.0 in water occurred in areas covered with plants with little diel variation (McDonald and Wolverton, 1980; Reddy, 1981) which suggests that NH_3-N volatilization is minimal in these systems.

Decomposition of Plant Tissue in Freshwater

Decomposition of plant tissue in a freshwater habitat commonly occurs in two stages. The first stage was attributed to leaching of the more soluble plant constituents while the second stage was microbial-controlled degradation (Boyd, 1970b, Hunter, 1976, Godshalk and Wetzel, 1978a, Howard-Williams et al., 1983).

Otsuki and Wetzel (1974) reported a rapid leaching loss of dissolved organic matter regardless of conditions of aerobiosis or whether plants were fresh or freeze-dried. Hill (1979) concluded that rapid leaching of soluble material accounted for a 21 to 50% dry weight loss of aquatic macrophytes during the first 8 days of incubation. Leaching was established as the major process in the decomposition of eelgrass and total loss of organic matter by leaching accounted for 82% of dried leaves and 65% of fresh leaves (Harrison and Mann, 1975). Leaching rates appeared to be independent of temperature (Carpenter, 1980).

Potassium, Na, Mg, and Ca have all been reported as being rapidly lost during the early leaching phase of plant decomposition (Boyd, 1970b, Davis and van der Valk, 1978, Puriveth, 1980). Carpenter (1980) found that the higher the initial P concentration, the more rapid was P leaching.

The second stage of decomposition is attributed to biological processes. Microbial-controlled decomposition was influenced by temperature (Carpenter, 1980, Puriveth, 1980), pH (Sompongse, 1982), available O_2 (Godshalk and Wetzel, 1978a), and available nutrients (Carpenter and Adams, 1979; Puriveth, 1980). Godshalk and Wetzel (1978a) found that the presence of O_2, regardless of temperatures of 10 or 25°C, permitted rapid degradation of dissolved and particulate organic matter. Decomposition of water hyacinth was found to be faster under aerobic than anaerobic conditions (Reddy and Sacco, 1981). However, Sompongse (1982) determined that aeration did not have a measurable effect on rate of plant decomposition and Nichols and Keeney (1973) reported a more rapid decomposition rate under non-aerated conditions compared to aerated conditions. Ogwada et al. (1984) found that approximately the same amounts of N and P were released from decaying plant tissue under aerobic or completely anoxic conditions, but the extent of nutrient release was dependent on water temperature.

The changes in plant C and N composition or concentration during decomposition have received considerable attention. Build-up of microbial biomass on decaying plant tissue caused a loss in the C content while increasing the N content which resulted in a decrease in the C/N ratio (De La Cruz and Gabriel, 1974, Odum and Heywood, 1978; Sill, 1979). Godshalk and Wetzel (1978b) found that lignin was very resistant to decomposition while the other structural carbohydrates gradually decreased with time.

Nitrogen was a limiting factor in decomposition of several aquatic plants (Nichols and Keeney, 1973; Almazan and Boyd, 1978, Godshalk and Wetzel, 1978b, Carpenter, 1980). Decay rates were correlated both to initial N content and to C/N ratios (Godshalk and Wetzel, 1978b, Carpenter and Adams, 1979, Ogwada et al., 1984).

Particle size also influenced decomposition. Generally, the rate of decomposition increased as the particle size decreased (Fenchel, 1970, Hargrave, 1972, Gosselink and Kirby, 1974). Harrison and Mann (1975) reported that a reduction in size of leaf material from 2 to 4 cm to <1 mm doubled the rate of organic matter loss.

Boyd (1970b) and Odum and Heywood (1978) concluded that submerged leaves decomposed more rapidly than those placed upon the water surface or suspended in air. Nichols and Keeney (1973) found more rapid dry weight loss under aerated conditions in sediment-water systems than in water only. They attributed this difference to an additional supply of N from the sediments.

Anaerobic Digestion

The second component of the integrated "biomass for energy" system was anaerobic digestion of plant biomass for methane production. Anaerobic digestion is a biological process in which organic matter, in the absence of oxygen, is converted to methane and carbon dioxide (Toerien and Hattingh, 1969).

During the process of anaerobic digestion, waste organic C was stabilized by the nearly complete microbial fermentation of carbohydrates resulting in a reduction of volatile solids (Miller, 1974). Anaerobically digested sewage sludges were considered more stable to microbial degradation than were aerobically digested sludges (Sommers, 1977).

Processes which regulated anaerobic digestion include hydrolysis of polymers, the dissimilation of starting substrates to the level of acetic acid, and the conversion of acetic acid to CH_4 and CO_2 (Mah et al., 1977). Factors which influenced anaerobic digestion include pH and temperature changes. All methanogens were reported to be strict anaerobes with an optimum pH of 6.7 to 7.4 (Bryant, 1979). The optimum temperature range was 30 to 35°C (Souse, 1981).

Water hyacinth biomass could be anaerobically digested to produce methane. Hanisak et al. (1980) found average methane yields of 0.24 L g^{-1} volatile solids (VS) of shredded water hyacinth in 162 L digesters. Chynoweth et al. (1983) reported methane yields of 0.19 and 0.28 L g^{-1} VS for water hyacinths and a 3:1 blend of water hyacinths:domestic sewage sludge, respectively in 5 L digesters. Shiralipour and Smith (1984) reported average methane yields of 0.32 and 0.47 L g^{-1} VS water hyacinth shoot and root samples, respectively, in a bioassay test of 100 ml culture volume. They concluded that the addition of N to growth media for water hyacinth production increased methane yields.

The nutrients in the biomass are recovered in the waste material after the digestion process. The recovered nutrients are distributed between the effluent and the digested biomass sludge. The total N recovery was usually 100% after digestion, but much of the organic N was converted to NH_4^+-N (Hashimoto et al., 1980, Field et al., 1984). Most of the K (Field et al., 1984) and Na (Atalay and Blanchar, 1984) were solubilized and remained in the digester effluent.

The digestion process increased the sorption of some nutrients (P, Ca, and Mg) by the sludge fraction such that fewer were available for dilute acid extraction and perhaps for crop recovery (Field et al., 1984). Field et al. (1984) hypothesized that sorption may have been increased by particle surface area increases due to size reduction of solids.

Nitrogen Cycling During Anaerobic Digestion

Nitrogen transformations occurring during anaerobic digestion include 1) mineralization/immobilization, and 2) NH_3-N volatilization (Fig. 4).

The mineralization or immobilization of N depends on the initial N content of the biomass feedstock. The C/N ratio of the biomass is often used as a guideline for prediction of net mineralization or immobilization. The optimal C/N ratio of the added biomass was 30:1

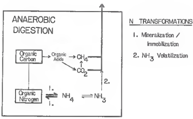

Figure 4. Nitrogen cycling during anaerobic digestion. Nitrogen and carbon transformations investigated during this study are indicated with larger arrows.

(Hughes, 1981). Bioconversion of biomass with a higher C/N ratio was limited by N. A lower initial C/N ratio resulted in mineralization of organic N during digestion. The C/N ratio of the digester effluent was lower than the C/N ratio of the fresh slurry because of the release of C as CO_2 and CH_4 (Hesse, 1981).

Anaerobic digestion of plant biomass resulted in high concentrations of NH_4^+-N in the digester (Hashimoto et al., 1980; Field et al., 1984). Ammonium was toxic to methogens at concentrations > 3.0 g l-1, regardless of pH (Hashimoto et al., 1980). Losses of NH_3-N through volatilization should be low in digesters operating at the optimum pH of 6.7 to 7.2 unless NH_4^+-N concentrations are high.

Waste By-Product Recycling

The final component of the integrated "biomass for energy" system was recycling of the waste by-product generated from the anaerobic digestion of plant biomass. The waste by-product from the anaerobic digester was screened to separate the digested biomass sludge from the effluent. The effluent was recycled in the water hyacinth biomass production system discussed earlier. Methane digester effluent contains high levels of NH_4^+-N (> 200 mg L^{-1}) which may inhibit plant growth. Dilution of the effluent is required before use in a water hyacinth production system. Optimum dilution of the effluent for maximum water hyacinth yields has not been established. Nitrogen cycling in a water hyacinth production system was presented earlier.

The sludge was added to soil as an organic amendment. A consequence of anaerobic digestion was a reduction of the readily decomposable C of the plant tissue during production of CH_4 and CO_2.

Anaerobically digested sludge was considered to be stable and resis further decomposition (Sommers, 1977)

Nitrogen Cycling in Soil Treated with Plant Residues

The land application of fresh or anaerobically digested plant biomass has significant implications on N cycling. Nitrogen transformations occurring after residue additions include 1) mineralization/immobilization; 2) microbial or plant assimilation; 3) nitrification, 4) denitrification, and 5) NH_3-N volatilization (Fig. 5)

Since most of the N in fresh or digested plant biomass is in organic form, the rate of mineralization becomes the rate limiting step for all transformations that follow. Mineralization or immobilization depends on the initial N concentration of the plant biomass as well as the composition of the C constituents. A low N content or a wide C/N ratio was associated with slow decomposition and rates of decomposition were proportional to lignin content (Alexander, 1977). A wide C/N ratio (> 30:1) favored N immobilization whereas a narrower ratio (< 20:1) resulted in N mineralization (Alexander, 1977)

Mineralization of N from anaerobically digested sewage sludges was reported to be affected by the rate of application (Ryan et al., 1973; Stark and Clapp, 1980). However, Epstein et al. (1978) found that irrespective of the amount of material (sewage sludge and sludge compost) applied, the percentage of added N mineralized remained essentially constant.

The mineralization of NH_4^+-N from organic N is accompanied by microbial assimilation or plant uptake. In aerobic environments the NH_4^+-N was quickly converted to NO_3^--N (nitrification) which could also be

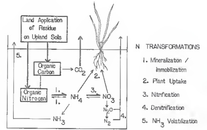

Figure 5 Nitrogen cycling in soil treated with plant residues Nitrogen and carbon transformations investigated during this study are indicated with larger arrows

assimilated by microbes or plants (Ryan et al., 1973). A high organic loading rate may result in O_2 depletion during decomposition which promotes the denitrification process (Epstein et al., 1978, Hsieh et al., 1981b)

Decomposition of Plant Residues in Soil

Decomposition of plant residues in soil occurs in two stages. The first stage was attributed to loss of the easily decomposable labile fraction which was followed by the second stage of slow decomposition of a resistant residual fraction (Shields and Paul, 1973, Reddy et al., 1980) Both stages were thought to be controlled by two simultaneously occurring superimposed first-order kinetic reactions (Sinha et al., 1977)

Fresh and anaerobically digested plant biomass differ widely in their chemical composition Anaerobic digestion converts most of the easily-decomposable plant C constituents into CH_4 and CO_2. The digested biomass sludge has a higher lignin content and is more resistant to decomposition There is little information available on decomposition of anaerobically digested plant biomass added to soil. However, the rates and the factors which influence decomposition of fresh plant biomass added to soil have been well established

Tenny and Waksman (1929) concluded that water-soluble organic substances were first to be decomposed in the soil, followed by hemicelluloses and at the same time, or immediately after, cellulose. Lignin was very resistant to decomposition and may even delay the disintegration of cellulose or hemicellulose because of the structural proximity of these C constituents in the cell wall (Tenny and Waksman, 1929; Peevey and Norman, 1948; Berg et al., 1982).

Application rates were shown to have insignificant effects on rate of fresh plant biomass decomposition (Jenkinson, 1965, Nyhan, 1975). However, several studies suggested that small amounts of fresh or anaerobically digested plant biomass decomposed more rapidly than large quantities (Broadbent and Bartholomew, 1948, Jenkinson, 1971; Atalay and Blanchar, 1984)

Miller and Johnson (1964) found an increasing rate of CO_2 production with increasing moisture content up to a tension of 0.05 to 0.015 MPa and then a decreasing rate with further increases in tension. They concluded that maximum biological activity could be expected at the lowest tension when aeration was sufficient. Orchard and Cook (1983) found a log-linear relation between water potential and microbial activity in the range of 0.005 to 0.5 MPa

Sain and Broadbent (1977) concluded that low temperatures influenced decomposition rate more than excessive moisture. However, Nyhan (1976) found a pronounced decrease in rates of C loss with an increase in soil water tension even when temperature (10°C) was limiting microbial activity. Miller (1974) determined that soil temperature was the major factor influencing the rate of decomposition of anaerobically digested sewage sludge

Decomposition was generally considered to be initially slower in acid than neutral soil (Jenkinson, 1971). Addition of organic material altered the pH of a soil, particularly when the amount added was large relative to the amount of native organic matter present (Jenkinson, 1966). Atalay and Blanchar (1984) found that addition of anaerobically digested biomass sludge to soil increased the pH from 5.5 to 7.6 and they attributed this to a limestone buffer used during the digestion process

Jenkinson (1965, 1971), using different plants and soils, determined that the proportion of added plant C retained in the soil under different climatic conditions was remarkably similar over time. Generally, one-third of the added plant C remained after one year, falling to one-fifth after 5 years.

Ateley and Blanchar (1984) determined that anaerobically digested biomass sludge decomposed rapidly in soil as evidenced by nearly 40% of the C added evolved as CO_2 during 100 days of decomposition. However, Miller (1974) concluded that anaerobic digested sewage sludge was resistant to further decomposition with a maximum of 20% of the added C evolved as CO_2 during a 6-month incubation. Terry et al. (1979) found that 26 to 42% of anaerobically digested sewage sludge C was evolved as CO_2 during incubation. Generally, the majority of the CO_2 produced in incubation studies was evolved in the first 30 days (Miller, 1974, Terry et al., 1979; Ataway and Blanchar, 1984).

Other soil properties influenced by plant biomass additions included increasing water-holding capacity, CEC, and electrical conductivity (Stark and Clapp, 1980, Ateley and Blanchar, 1984). Epstein et al. (1976) found levels of salinity and chloride in sewage sludge applied to soils increased to a level which may effect salt-sensitive plants.

Conclusions

Although information is available on N cycling for each component of the system, no attempt has been made to follow N transformations in an integrated "biomass for energy" system. Evaluation of N cycling was chosen because N is often identified as a limiting factor for plant growth and is used to establish loading rates in the disposal of solid and liquid waste.

Plant uptake was established as a major N removal process during water hyacinth biomass production (Reddy and Sutton, 1984). However, the role of water hyacinth detritus as a N source or sink has not been established. Methane yields during anaerobic digestion of water hyacinth were enhanced with increasing N content (Shiralipour and Smith, 1984). However, N mineralization rates were not investigated. Limited information was available on disposal or utilization of digester effluent or sludge from anaerobically digested plant biomass (Hanisak et al., 1980, Atalay and Blanchar, 1984). The overall objective of this research was to integrate the three components of biomass production, anaerobic digestion of biomass, and digester waste recycling with respect to N cycling.

WATER HYACINTH BIOMASS AND DETRITUS PRODUCTION

Water hyacinth is one of the most productive aquatic macrophytes found throughout the tropical and subtropical regions of the world. The plant has been used extensively for treatment of nutrient-enriched waters and currently there are a number of wastewater treatment systems in the U.S. utilizing water hyacinths for secondary and tertiary treatment (Cornwell et al.,1977, Dinges, 1978, Wolverton and McDonald, 1979; Reddy et al., 1985).

Water hyacinth productivity has been evaluated in natural and nutrient-enriched waters. Growth rates of 2 to 29 g dry wt m^{-2} day^{-1} were reported for plants growing in natural waters of central and south Florida (Yount and Crossman, 1970, DeBusk et al., 1981). A wide range of productivity (5 to 42 g dry wt m^{-2} day^{-1}) was recorded for plants cultured in nutrient-enriched waters (Hanisak et al., 1980; Reddy and Bagnell, 1981; Reddy, 1984). Maximum growth rates provided an average of 52 and a maximum of 64 g dry wt m^{-2} day^{-1} for plants cultured under nutrient-nonlimiting conditions (Reddy and DeBusk, 1984).

Plant detritus (dead and decaying plant debris) is an integral part of water hyacinth mats. Detritus is usually derived from natural aging of plants, biological or chemical control, and frost damage. Decomposition of detritus releases nutrients which can be subsequently utilized by water hyacinths. Information on water hyacinth productivity was extensive (Reddy et al., 1983), but research on detritus production and its role as a nutrient sink or source was limited.

DeBusk et al. (1983) measured detritus production in harvested and nonharvested water hyacinth based sewage treatment systems. Detritus production in both systems averaged 2 g dry wt m^{-2} day^{-1}. More than 80% of the detritus consisted of root material. The bulk of the standing crop detritus remained trapped in the floating plant mat. However, this study did not reveal the potential of detritus as a nutrient input to the water hyacinth ponds.

The objectives of this study were to 1) measure productivity and detritus (shoot and root) production of water hyacinths grown in eutrophic lake water with and without added nutrients and 2) determine the potential of detritus as a nutrient source or sink to the ponds.

Materials and Methods

The study was conducted in two reservoirs located at the Central Florida Research and Education Center research farm near Lake Apopka in Zellwood, Florida. The reservoirs were constructed with 2.0 m high levees of a Lauderhill organic soil (Lithic medisaprists) and with bottoms composed of calcareous clay. The water depth was 60 cm and the dimensions of the reservoirs were 7.6 m by 61 m (total surface area of 465 m^{2}). Both reservoirs were filled with water from nearby Lake Apopka, and were sectioned into four equal areas for replication and stocked with water hyacinths.

A total of eight 0.25 m^{2} cages (Vexar mesh screen connected to 5 cm diameter PVC pipe) were stocked with water hyacinth at an initial density of 16 kg (fresh wt) m^{-2}. The cages were placed within the four replicated areas of each reservoir. Each cage was lined with 1.00 mm fiberglass screen to retain any detritus dislodged from the plant mat. The fiberglass screen was positioned 15 cm below the PVC frame to allow normal waterflow within the root mat.

One reservoir was fertilized monthly by broadcasting a 10-4-10 fertilizer to add 100 kg N ha^{-1} from October 1981 to February 1982, and 50 kg N ha^{-1} from March 1982 to September 1982. The change in fertilizer rate was due to an excess of 10 mg N L^{-1} found in reservoir water several days after fertilization during winter. The second reservoir contained Lake Apopka water with no added nutrients and served as the control. Both reservoirs were drained and refilled with Lake Apopka water monthly (24 hr prior to plant sampling and fertilization).

Plant productivity and detritus production were monitored at monthly intervals for one year. The cages were removed from each section, drained for 5 min, and weighed. The plant material was divided into healthy plants and detritus. Detritus was defined as that collected from the fiberglass screen and dead shoot and root material remaining within the plant mat. Dead shoots were defined as material visibly devoid of chlorophyll. Three plants were removed for analyses and the cages were restocked to the initial plant density and placed in the reservoirs.

The shoot and root lengths were recorded for each plant sample and separated for dry weight ratios. The plant tissue and detritus were oven dried at 70°C, weighed, and ground to pass a 0.84 mm Wiley Mill screen. All plant and detritus samples were analyzed for total Kjeldahl nitrogen (TKN) using a modified micro Kjeldahl procedure (Nelson and

Summers, 1983). Solar radiation and high and low daily temperatures were recorded. The results were statistically analyzed for a randomized block design with the fertilized and control reservoirs as treatments.

Results and Discussion

The weekly averages of daily maximum and minimum air temperatures and solar radiation are shown in Fig. 6. Maximum temperatures ranged from 21.9°C during January to March and 36.5°C during July to September. Minimum temperatures for these time periods were 8.2°C and 20.3°C, respectively. Maximum and minimum temperatures for the rest of the year were similar (high= 30°C, low= 13.5°C). Maximum and minimum solar radiation occurred from April to September and from November to March, respectively.

The monthly averages of daily primary productivity and detritus production of water hyacinth are presented in Fig. 7. Maximum daily water hyacinth productivity during this study was observed in August for the fertilized reservoir (28.3 g dry wt m^{-2} day^{-1}) compared to June for the control reservoir (14.7 g dry wt m^{-2} day^{-1}). Detritus production remained fairly consistent with time for both reservoirs. Detritus production in the fertilized reservoir increased noticeably in September when plant productivity began to decline. The average daily detritus production in the fertilized reservoir was 3.7 g dry wt m^{-2} day^{-1} compared to 3.5 g dry wt m^{-2} day^{-1} in the control reservoir. DeBusk et al. (1983) found that detritus production occurred at a relatively constant rate regardless of harvested or nonharvested conditions.

Monthly data for the plant parameters have been summarized by seasons: 1) autumn (October, November, and December), 2) winter

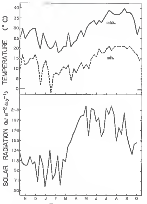

Figure 6. Weekly averages of daily temperatures and solar radiation.

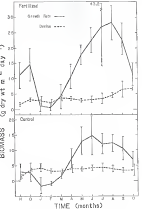

Figure 7. Monthly averages of daily primary productivity and detritus production.

(January, February, and March; 3) spring (April, May, and June), and 4) summer (July, August, and September). The seasons were chosen to coincide with changes in water hyacinth productivity and temperature and solar radiation changes. The autumn season combines data for November and December of 1982 and October of 1983.

The effects of temperature and solar radiation were evident since the lowest net productivity occurred during winter and the highest during spring and summer (Table 1). The net primary productivity in winter for both reservoirs was lower than the production of detritus. Reddy and Bagnall (1981) reported that at average temperatures of 10°C, productivity of water hyacinth approached zero. The majority of the detritus during winter came from the destruction of aerial shoots caused by freezing temperatures. Although a majority of the aerial shoots were destroyed, the plants survived, and noticeable gains in dry weight began in March.

There were significant differences in yields between seasons and reservoirs. Seasonal yields ranged from 1.9 to 23.1 Mg (dry wt) ha^{-1} for the fertilized reservoir and -0.2 to 10.2 Mg ha^{-1} for the control reservoir. Over 75% of the biomass production occurred during spring and summer for both reservoirs. Differences in detritus production were not significant between reservoirs or between seasons. Although the annual yield of water hyacinth in the fertilized reservoir was double that of the control reservoir, the detritus production in both reservoirs was similar (Table 1). There were significant differences in the shoot/root dry weight ratios between reservoirs, but not between seasons (Table 1). The average shoot/root dry weight ratio was 1.93 (1.64 to 2.48) for the fertilized reservoir and 1.14 (0.78 to 1.87) for the control reservoir.

Table 1. Seasonal water hyacinth yield and detritus production.

	Fertilized reservoir			Control reservoir		
Season	Yield	Detritus	S/R §	Yield	Detritus	S/R
	---Mg ha^{-1}----			---Mg ha^{-1}---		
Autumn (78) †	9.5	2.8	1.85	3.3	2.2	1.67
Winter (80)	1.9	2.7	1.75	0.2	3.2	1.25
Spring (84)	14.7	2.7	1.64	9.7	3.3	0.96
Summer (88)	23.1	4.1	2.46	10.2	3.3	0.79

Test of Significance ‡	Yield	Detritus	S/R
Season	**	NS	NS
Month (season)	NS	NS	NS
Reservoir	**	NS	**

† Number of days in season.
‡ Significant at 0.01 level (**) or not significant (NS)
§ S/R = Shoot/root dry wt ratio

Shoot and root lengths were similar for plants in both reservoirs during autumn and winter (Table 2). During spring, the water hyacinth root lengths were shorter and shoot lengths longer in the fertilized reservoir compared to the plants in the control reservoir. An interesting development in plant morphology was the dislodging of practically the entire root system from plants in the fertilized reservoir beginning in March after daily temperatures began to increase. The majority of plants were typified by a small root system compared to plants in the control reservoir. Some root dislodging was noticed in the control reservoir during spring and summer but was not as characteristically uniform as in the fertilized reservoir. Root lengths in the fertilized reservoir began to increase during summer, but the shoot lengths were double those in the control reservoir.

Under nutrient-limiting conditions, water hyacinths produce a large volume of root material presumably to increase their nutrient absorption capacity. With nutrient-enriched media, water hyacinth use more photosynthetic energy in shoot production. Cornwell et al. (1977) measured shoot lengths in excess of 1 m in wastewater media. Penfound and Earle (1948) recorded root lengths of 0.1 to 1 m. Maximum shoot length recorded during this study was 55 cm during summer for fertilized plants compared to 28 cm during summer for control plants.

The plant tissue N content is shown in Fig. 8. Fertilization resulted in increases in shoot, root, and detritus N content compared to plants in the control reservoir. Maximum tissue N content for fertilized plants occurred during winter when plant productivity was low. The increase in plant productivity in spring and summer diluted the N content of the tissue although total N assimilation by the plants

Table 2 Seasonal water hyacinth shoot and root lengths

	Fertilized reservoir		Control reservoir	
Season	Shoots	Roots	Shoots	Roots
	------------	----------cm--	------------	------------
Autumn (78)[†]	39.6	21.5	35.7	22.9
Winter (90)	25.2	14.2	23.3	20.2
Spring (84)	26.6	9.9	18.8	14.8
Summer (88)	54.9	21.3	27.8	27.1
Test of significance[‡]	Shoot length		Root length	
Season	**		**	
Month (Season)	NS		**	
Reservoir	*		**	

[†] Number of days in Season

[‡] Significant at 0.05 (*) or 0.01 (**) level, or not significant (NS)

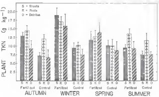

Figure 8 Seasonal plant tissue nitrogen content.

was much greater during this time period (Table 3). Plant tissue N content remained nearly consistent with time in the control reservoir and root tissue N content generally exceeded that of the shoot tissue (Fig. 8).

Seasonal N assimilation by water hyacinth ranged from 34 to 242 kg N ha^{-1} for plants in the fertilizer reservoir and from <0 to 104 kg N ha^{-1} for plants in the control reservoir (Table 3). There were significant differences between seasons and reservoirs in water hyacinth N assimilation. The detritus N content was significantly greater for fertilized than control plants, but there were no significant differences in detritus N content between seasons.

Data on mass balance of N in both reservoirs are shown in Table 4. Nitrogen input from the lake was 238 kg N ha^{-1} with 89% of the N in the organic fraction. Total amount of fertilizer applied during the study period was 781 kg N ha^{-1}, with NH_4^+-N, NO_3^- N, and organic N representing 55, 30, and 15% of total fertilizer applied, respectively.

The total N assimilated by water hyacinth (live plants and detritus) was 720 and 325 kg ha^{-1} yr^{-1} for fertilized and control reservoirs, respectively (Table 4). Annual net N loading by detritus was 148 and 92 kg ha^{-1} for fertilized and control reservoirs, respectively (Table 4). Maximum detritus N loading occurred during winter for the fertilized reservoir and during spring for the control reservoir. This corresponded to the time of root dislodging from plants in the two reservoirs.

The annual net N immobilized by detritus represented 21 and 28% of the total N removed by water hyacinth in the fertilized and control reservoirs, respectively. DeBusk et al. (1983) concluded that

Table 3 Seasonal nitrogen uptake by water hyacinth and detritus

	Fertilized reservoir		Control reservoir	
Season	Water hyacinth	Detritus	Water hyacinth	Detritus
	------------------	------kg N ha^{-1}----	------------------	
Autumn (78) [†]	127.8	28.2	30.9	14.6
Winter (90)	33.9	42.4	-1.2	23.7
Spring (84)	167.7	37.5	104.3	29.1
Summer (85)	242.4	39.8	98.7	24.4

Test of significance [‡]	Water hyacinth	Detritus
Season	**	NS
Month (season)	NS	*
Reservoir	**	**

[†] Number of days in season

[‡] Significant at 0.05 (*) or 0.01 (**) level or not significant (NS)

immobilization of N as plant detritus was 3 and 33% of standing crop assimilation for harvested and non-harvested water hyacinth plants, respectively. However, they did not include plant detritus trapped within the water hyacinth mat.

The annual N assimilation by water hyacinth is low compared to N removal rates reported for plants growing in nutrient-enriched waters. Reddy et al. (1985) found annual N removal rates of 1726 and 1193 kg N ha^{-1} yr^{-1} for water hyacinths growing in primary and secondary sewage effluent, respectively. Rogers and Davis (1972) concluded that water hyacinths could remove 2500 kg N ha^{-1} yr^{-1} if maximum growth could be sustained. Sato and Kondo (1981) measured a maximum removal rate of 4782 kg N ha^{-1} yr^{-1} for plants growing in a nutrient medium. The low annual N assimilation reported in the present study was due to low rates of fertilization.

Plant uptake played a major role in removing N in both the reservoirs (Table 4). A large portion of lake water N was present as organic N, which was not readily available to plants. In both reservoirs, plants derived N from mineralization of lake water organic N, N release from underlying sediments, and mineralization of organic N in detritus. In the fertilized reservoirs, plants also derived N from the fertilizer N applied. Nitrogen assimilation by water hyacinth from the added fertilizer was calculated as follows: (Total N assimilation by plants in the fertilized reservoir - Total N assimilation by plants in the control reservoir / Total fertilizer N added) * 100.

About 51% of the added fertilizer N was taken up by the plants in the fertilized reservoir, and the remaining 49% may have been lost through denitrification. Reddy et al. (1982) observed a reduction of 7%

Table 4 Nitrogen balance for the two reservoirs.

	Fertilized reservoir	Control reservoir
	---------kg ha^{-1}-----------	
	Nitrogen added	
Fertilizer		
NH_4^+-N	430	--
NO_3^--N	234	--
Organic N	117	--
Total	781	
Lake water		
NH_4^+-N	13	13
NO_3^--N	12	12
Organic N	213	213
Total	238	238
Total added	1018	238
	Nitrogen removed	
Water hyacinth		
Shoots	354	85
Roots	218	137
Detritus	148	92
Total	720	325
Reservoir water		
NH_4^+-N	13	8
NO_3^--N	13	5
Organic N	167	156
Total	193	168
Total N accounted	913	494

to 81% of the agricultural drainage effluent NO_3^--N and NH_4^+-N in 3.6 days in a reservoir containing water hyacinths. DeBusk et al. (1983) calculated that 45% of the N removed from wastewater was immobilized in water hyacinth standing crop and detritus. About 30% of the fertilizer N was added as NO_3-N, which could be potentially lost due to denitrification. Since water hyacinth plants prefer NH_4^+-N over NO_3^--N (Reddy and Tucker, 1983), the majority of the plant N uptake probably came from NH_4^+-N added through fertilizer. The role of underlying sediment in the immobilization/mineralization, and denitrification of N from these systems needs further investigation.

Total N recovery in the fertilized reservoir was about 90%, and plant uptake represented about 71% of total N inputs. In the control reservoir, total N recovery was higher than the N inputs. Plants removed 325 kg N ha^{-1}, as compared to 238 kg N ha^{-1} added. Release of N from sediment or mineralization of N during decomposition of detritus may account for the higher N recovery compared to total N inputs. Ogwada (1983) found a yearly average of 150 ± 34 kg KCl-extractable inorganic N ha^{-1} sediment using monthly sediment N concentrations of the same reservoirs.

Conclusions

Primary productivity of water hyacinths was influenced by ambient air temperature, solar radiation, and nutrient composition of the culture medium. Net detritus production (total detritus - detritus lost through decomposition) was relatively constant throughout the year and represented 3.5 to 14.0% of the total standing crop. Detritus plant tissue of the fertilized reservoir contained higher tissue N, compared to the detritus in the control reservoir. Fertilization and increases in ambient air temperature resulted in dislodging of root biomass.

Net N loading from detritus was 92 to 148 kg N ha^{-1} yr^{-1}, which is potentially available upon decomposition. The N immobilized by detritus represented 21 and 28% of the total N removed by water hyacinths in the fertilized and control reservoirs, respectively.

Approximately 51% of the added fertilizer N was assimilated by plants. The remaining 49% may have been lost through denitrification. Total N recovery was nearly 90% in the fertilized reservoir. More N was accounted for in the control reservoir than was added. Release of N from the sediment or mineralization of N during decomposition of detritus may account for the additional N recovery.

EFFECT OF DETRITUS ON NITROGEN TRANSFORMATIONS IN WATER HYACINTH SYSTEMS

Plant detritus (dead and decaying plant debris) is an integral part of water hyacinth mats and comprises 3 to 14% of the total biomass (see p 39) It is usually derived from natural aging of plants, biological or chemical control, and frost damage The addition of detritus to an aquatic system influenced several C and N transformations (Fenchel and Jorgensen, 1977)

Nitrogen is present as NH_4^+-N, NO_3^--N, and organic N in water media available for water hyacinth production Organic N predominates in most water media and is not readily available for plant assimilation Water hyacinths were efficient users of inorganic N and plant assimilation was a major process of N removal in aquatic systems containing water hyacinth (Reddy and Sutton, 1984) Other N transformations in aquatic systems resulting in removal of NO_3^--N or NH_4^+-N include microbial assimilation, nitrification/denitrification, and NH_3-N volatilization (Keeney, 1973, Bouldin et al , 1974) Addition of detritus significantly alters the rates of these processes

Mineralization or immobilization of N occurs during decomposition of detritus in water and sediment Decomposition of detritus and subsequent N release was found to be related to C/N ratio, initial N and fiber contents (De La Cruz and Gabriel, 1974, Godshalk and Wetzel, 1978b, Odum and Heywood, 1978, Ogwada et al , 1984)

A dense cover of floating water hyacinth depleted dissolved O_2 of the underlying water, thus creating anaerobic conditions (Boyd, 1970; McDonald and Wolverton, 1980, Reddy, 1981). Decomposition of plant detritus also consumed O_2 (Nichols and Keeney, 1973, Rai and Munshi, 1979). Anaerobic conditions may restrict nitrification and favor denitrification, which may proceed within the water hyacinth mat, in the water column, or in the underlying sediment. Detritus also provides energy source for denitrification. Denitrification occurred primarily in the underlying sediment and the rate depended on NO_3^--N diffusion from the water column to the sediment (Engler and Patrick, 1974; Reddy and Graetz, 1981).

Volatilization becomes increasingly important as the water pH increases. The partial pressure of NH_3 N in equilibrium with a solution of NH_3-N increased rapidly in a pH range of 8.5 to 10.0 (Bouldin et al., 1974). A pH of 7.0 in water occurred in areas covered with water hyacinth with little diel variation (McDonald and Wolverton, 1980, Reddy, 1981) which suggests that NH_3 N volatilization is minimal in areas covered with plants.

The relative role of N assimilation by water hyacinth on total N removal from reservoirs was investigated by Reddy (1983). Approximately 40% of added $^{15}NH_4^+$-N or $^{15}NO_3^-$-N was assimilated by plants. Less than 10% of the added ^{15}N was found in the surface sediment layer. Over 40% of the added ^{15}N was unaccounted for.

Information on the role of detritus in aquatic systems on immobilization or mineralization of inorganic N is limited. The overall objective of this study was to determine the effect of detritus on selected N transformations in water columns with and without water hyacinths. Specifically, the objectives were 1) to determine the regulatory function of detritus on dissolved O_2 and pH of water and 2) to determine the influence of detritus on the fate of $^{15}NO_3^-$-N and $^{15}NH_4^+$-N in sediment- water-plant systems.

Materials and Methods

Two greenhouse studies were conducted to evaluate the effect of detritus on the fate of ^{15}N labeled NO_3^--N or NH_4^+-N in water with and without water hyacinth plants. Treatments evaluated were: 1) with and without underlying sediment, 2) with and without water hyacinth plant cover, and, 3) three rates of added water hyacinth detritus. There were 24 tanks in each study having dimensions of 50 cm * 50 cm * 25 cm depth. Twelve of the 24 tanks contained a 2.5 cm sediment layer (1.875 kg soil). The sediment was a Lauderhill organic soil (Lithic medisaprists) collected at the Central Florida REC research farm in Zellwood, Florida. The soil was air-dried and passed through a 2 mm sieve. Fifty liters of tap water were added to sediment tanks to obtain a 20 cm water depth.

The greenhouse studies were initiated after sediment/water equilibration of 1 week. A nutrient medium (a modified 10% Hoagland's solution) was added to all tanks to obtain nutrient concentrations of: $^{15}NH_4^+$-N or $^{15}NO_3^-$-N = 20.0 mg L^{-1}; K = 23.5 mg L^{-1}, PO_4-P = 3.1 mg L^{-1}, Ca = 20.0 mg L^{-1}, Mg = 4.8 mg L^{-1}, SO_4-S = 6.4 mg L^{-1}, Fe = 0.6 mg L^{-1} and micronutrients. Micronutrients were applied through commercially available liquid fertilizer (Nutrispray Sunniland, Chase and Co., Sanford, Florida) to obtain concentrations of 0.2 mg Cu L^{-1}; 1.5 mg Mn L^{-1}; 0.04 mg B L^{-1}, and 0.02 mg Mo L^{-1}.

Water hyacinth detritus (shoot and root material) was added at ne rates of 0, 100, and 400 mg C L^{-1}. The detritus was chopped manually to lengths of 2 cm. The detritus for treatments with added $^{15}NO_3^-$-N was collected from a natural water hyacinth stand in Zellwood, Florida and had an initial N content of 5.6 mg g^{-1} dry tissue. The detritus for treatments with added $^{15}NH_4^+$-N was collected from a water hyacinth stand located in a wastewater stabilization pond at the University of Florida wastewater treatment plant in Gainesville, Florida and had an initial N content of 23.1 mg g^{-1} dry tissue.

Water hyacinths, at an initial density of 10 kg (fresh wt) m^{-2}, were placed in 12 of 24 tanks. The plants were collected from the University of Florida's Sivens Arm research reservoirs in Gainesville, Florida. The plants were clipped of dead tissue and rinsed with tap water prior to placement in tanks.

The disappearance of added inorganic N was determined by collecting water samples at 0, 1, 2, 3, 4, 8, 15, 28 days and measuring NH_4^+-N, NO_3^--N, and total Kjeldahl N (TKN). The changes in water hyacinth fresh weight were measured weekly. Plant samples and detritus were analyzed for TKN. The sediment was characterized for organic and inorganic N prior to and at the conclusion of each study. Fifty grams (dry wt) of moist sediment samples were extracted with 2 M KCl and analyzed for NH_4^+-N and NO_3^--N. Sediment samples were air dried, ground by mortar and pestle, and analyzed for TKN. The inorganic N for all samples was determined by steam distillation (Keeney and Nelson, 1982). The TKN of water, plant, and sediment samples were determined by micro-Kjeldahl procedures (Nelson and Sommers, 1972, 1973; 1975). The ^{15}N analyses on water, sediment, plant and detritus samples were conducted using a Micro Mass 602 spectrometer

Water pH (Orion Model 404 Specific Ion Meter), dissolved O_2 (Yellow Springs Instrument Model 54 O_2 meter) and temperature were measured every other day. Electrical conductivity (Hach Mini Conductivity Meter) was measured weekly.

Results and Discussion

Effect of Detritus on Water Dissolved O2

The dissolved O_2 concentrations of water with added $^{15}NO_3^-$-N and $^{15}NH_4^+$-N are shown in Figs. 9 and 10, respectively. Dissolved O_2 concentrations remained < 5 mg L^{-1} in water having plant cover but lower dissolved O_2 concentrations were recorded as the rate of detritus increased. This reflected increasing microbial O_2 demand for respiratory functions with increasing C source (Penchal and Jorgensen, 1977). For water without plant cover (open water), the dissolved O_2 concentrations were scattered more with time. The dissolved O_2 measurements were taken between 2:30 and 3:30 pm and should represent near maximum concentrations on a diurnal basis (Heweler, 1972). The increased dissolved O_2 concentrations of open water were due to an increased rate of photosynthesis by algae during the day compared to respiration (Reddy, 1981).

Generally the effect of decreasing dissolved O_2 concentrations with increasing detritus was seen for open water with or without sediment. Dissolved O_2 concentrations were generally lower in open water with sediment compared to open water without sediment. Nichols and Keeney (1973) reported lower dissolved O_2 concentrations for sediment-water systems than water only. Although detritus appeared to have a role in O_2 dynamics in water, plant cover was the primary regulator

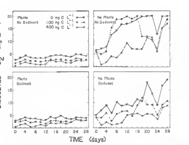

Figure 9 Dissolved O_2 in sediment-water-plant systems with adde [illegible]

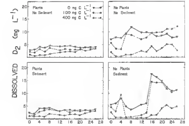

Figure 10 Dissolved O_2 in sediment-water-plant systems with added ammonium

Effect of Detritus on Water pH

The pH of water with added $^{15}NO_3^-$-N or $^{15}NH_4^+$-N is shown in Figs. 11 and 12, respectively. A fairly constant pH of ~ 7.0 was noted in water having plant cover and sediment regardless of detritus additions. The pH decreased in water with plant cover but without sediment. The decreasing pH was noted immediately for added $^{15}NH_4^+$-N and after 20 days for added $^{15}NO_3^-$-N. The decrease in pH was less as the detritus rate increased.

The immediate pH decrease in water with plants and added NH_4^+-N was probably due to production of H^+ during plant NH_4^+-N assimilation (Raven and Smith, 1976). The H^+ generated is actively exuded, partly in exchange for cations (Franco and Munns, 1982). Plant NO_3^--N assimilation occurs by exchange with another anion or by simultaneous cation assimilation to maintain ion equilibrium (Kirkby and Mengel, 1967; Mengel, 1974). The decreasing pH in water with plant cover but without sediment suggested that the underlying sediment had a buffering role in pH regulation.

The pH of water without plant cover were generally higher and more variable than water with plant cover. Reddy (1981) reported high mid day pH values in ponds where algal activity was high. Bouldin et al. (1974) found high pH values (> 8.5) for ponds containing submersed macrophytes during sunlight hours. The pH of open water was generally lower as the rate of detritus increased.

Effect of Detritus on Nitrogen Loss

Nitrogen loss from water with added $^{15}NO_3^-$-N or $^{15}NH_4^+$-N is shown in Figs. 13 and 14, respectively. Sediment or detritus had no apparent

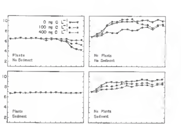

Figure 11. The pH of sediment-water-plant systems with added [illegible]

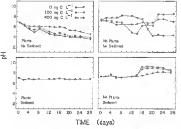

Figure 12. The pH of sediment-water-plant systems with [illegible]

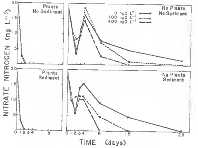

Figure 13 Nitrogen loss from sediment-water-plant systems with added nitrate.

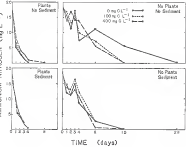

Figure 14 Nitrogen loss from sediment-water-plant systems with added ammonium

effect on rat[illegible] in water with plant cover. Nitrate and NH_4^+-N disappeared within 2 and 4 days, respectively, in water with water hyacinths.

Nitrogen loss in open water was influenced by the underlying sediment and detritus additions. Nitrate disappeared more rapidly in open water with sediment than without sediment. An increase in detritus resulted in a more rapid NO_3^--N loss in water with or without sediment. A rapid decrease in NO_3^--N after 2 days was followed by an accumulation of NO_3^--N at 4 days for open water. Accumulation of NO_3^--N after 4 days in open water was probably due to rapid algal assimilation followed by turnover (death) of the algae and leaching of NO_3^--N from the dead algal cells. Surface algal mats developed in open water within 2 days.

Ammonium disappeared more rapidly in open water with sediment than without sediment. Apparently detritus did not influence NH_4^+-N loss in open water with sediment. However, detritus additions resulted in a more rapid NH_4^+-N loss in open water without sediment. Loss of NH_4^+-N followed by accumulation of inorganic N during the first 4 days was not as striking as seen for NO_3^--N.

Plant Nitrogen Assimilation

Total plant N assimilation and the contribution of added $^{15}NO_3^-$-N and $^{15}NH_4^+$-N to total N assimilation is presented in Tables 5 and 6, respectively. Plant N assimilation was always greater for water with underlying sediment. Part of the increased plant N assimilation in water with sediment was due to release of N from the sediment. Generally the contribution of added $^{15}NO_3^-$-N or $^{15}NH_4^+$-N to total plant N assimilation also decreased with increasing detritus. Mineralization of

Table 5 Total plant N and $^{15}NO_3$-N assimilation.

Treatment	Total uptake	Labeled	Other ‡
	----mg (% of added ^{15}N)----		
Without sediment			
0 mg C L^{-1} †	767 ± 86	652 (85)	115 (15)
100 mg C L^{-1}	1088 ± 87	675 (62)	413 (38)
400 mg C L^{-1}	1027 ± 268	606 (59)	421 (41)
With sediment			
0 mg C L^{-1}	1220 ± 29	720 (59)	500 (41)
100 mg C L^{-1}	1171 ± 235	656 (56)	515 (44)
400 mg C L^{-1}	1112 ± 163	567 (51)	545 (49)

† Carbon source was plant detritus

‡ Other N sources include sediment and detritus

Table 6 Total plant N and $^{15}NH_4^+$-N assimilation

Treatment	Total uptake	Labeled	Other[‡]
	------mg (% of added ^{15}N)------		
Without sediment			
0 mg C L^{-1}[†]	903 ± 44	822 (91)	81 (9)
100 mg C L^{-1}	768 ± 103	697 (91)	71 (9)
400 mg C L^{-1}	1133 ± 81	807 (71)	326 (29)
With sediment			
0 mg C L^{-1}	1390 ± 194	891 (64)	499 (36)
100 mg C L^{-1}	1255 ± 89	845 (67)	410 (33)
400 mg C L^{-1}	1629 ± 65	828 (51)	801 (49)

[†] Carbon source was plant detritus

[‡] Other N sources include sediment and detritus

detritus N was a potential N source for plant assimilation. Reddy (1983) found that 60 to 64% of total N assimilation by water hyacinths was derived from added ^{15}N, while 36 to 40% was derived from sediment and from decomposition of detritus.

The ^{15}N recovered by plant tissue (mg) was fairly consistent for added labeled fertilizer but plant $^{15}NH_4^+$-N uptake exceeded $^{15}NO_3^-$-N uptake. Water hyacinth appeared to be more efficient in utilizing NH_4^+-N than NO_3^--N (Reddy and Tucker, 1983). When water hyacinth growth is not restricted by climate, rapid assimilation of added inorganic N would be expected.

Nitrogen-15 Balance for Water Columns

A ^{15}N balance for water with added $^{15}NO_3^-$-N and $^{15}NH_4^+$-N is presented in Tables 7 and 8, respectively. Total ^{15}N recovery by water hyacinths ranged from 57 to 72% and 70 to 89% in water with added $^{15}NO_3^-$-N and $^{15}NH_4^+$-N, respectively. Reddy (1983) conluded that water hyacinth N assimilation accounted for only 40% of added $^{15}NO_3^-$-N or $^{15}NH_4^+$-N in a reservoir. Algal surface mats accounted for -8% of added $^{15}NO_3^-$-N and up to 15% of added $^{15}NH_4^+$-N. The algal surface mats represented a minor portion of total microbial N assimilation. Algal activity was noted in open water, and the four sides and bottom of the microcosm tanks were colonized by algae.

The ^{15}N associated with detritus was determined for water with added $^{15}NH_4^+$-N but not for added $^{15}NO_3^-$-N. Less than 10% of the added $^{15}NH_4^+$-N was immobilized by detritus in water with plant cover. Newly formed water hyacinth detritus from the plant cover was deposited during 28 days and accounted for 7% ^{15}N recovery in water without added detritus or sediment.

Table [illegible] Mass balance [illegible]

Treatment	Plant or algae	Sediment[‡] org	Sediment[‡] inorg	Water	Total	Unaccounted For
	---------------	% Recovery of ^{15}N---			-	------
		PLANTS				
Without sediment						
0 mg C L^{-1}[†]	65.2	--	---	ND[§]	65.2	34.8
100 mg C L^{-1}	67.5	—	—	ND	67.5	32.5
400 mg C L^{-1}	60.6	---	---	ND	60.6	39.4
With sediment						
0 mg C L^{-1}	72.0	3.2	0.4	ND	75.6	24.4
100 mg C L^{-1}	65.6	4.4	0.4	ND	70.4	29.6
400 mg C L^{-1}	58.7	3.8	0.8	ND	59.9	40.1
		NO PLANTS				
Without sediment						
0 mg C L^{-1}	ND	---	--	6.2	6.2	93.8
100 mg C L^{-1}	6.8	---	---	ND	6.8	93.2
400 mg C L^{-1}	8.2	---	---	ND	8.2	91.8
With sediment						
0 mg C L^{-1}	ND	11.4	0.4	5.1	16.9	83.1
100 mg C L^{-1}	8.1	10.1	0.4	2.0	20.6	79.4
400 mg C L^{-1}	8.4	4.9	0.5	0.5	14.3	85.7

[†] Carbon source was plant detritus.
[‡] Org, Inorg = organic and inorganic N, respectively
[§] ND = Not detectable

Table 8 Mass balance of added $^{15}NH_4^+$-N in sediment-water-plant systems.

Treatment	Plant or algae	Detritus	Sediment[‡]		Water	Total	Unaccounted For
			Org	Inorg			
	---------------------% Recovery of ^{15}N------------						
PLANTS							
Without sediment							
0 mg C L^{-1}[†]	82.2	7.3	--	---	2.3	91.8	8.2
100 mg C L^{-1}	69.6	3.6	--	--	5.8	79.0	21.0
400 mg C L^{-1}	80.7	7.1			3.8	91.6	8.4
With sediment							
0 mg C L^{-1}	89.1	ND[§]	4.3	2.6	1.0	97.0	3.0
100 mg C L^{-1}	84.5	1.7	3.0	2.9	1.5	93.6	6.4
400 mg C L^{-1}	82.8	3.9	3.3	3.5	1.5	95.0	5.0
NO PLANTS							
Without sediment							
0 mg C L^{-1}	ND	ND		-	9.6	9.6	90.4
100 mg C L^{-1}	ND	14.7	-	---	17.5	32.2	67.8
400 mg C L^{-1}	6.1	34.7	--	---	5.2	46.0	54.0
With sediment							
0 mg C L^{-1}	14.9	ND	10.2	7.1	2.3	34.5	65.5
100 mg C L^{-1}	15.0	5.9	11.0	8.8	5.8	46.5	53.5
400 mg C L^{-1}	11.1	26.2	3.9	6.9	3.8	51.9	48.1

[†] Carbon source was plant detritus

[‡] Org, Inorg = organic and inorganic N, respectively.

[§] ND = Not detectable

Detritus ^{15}N recovery in water without plant cover increased with increasing rate of detritus. This suggests that during periods of low water hyacinth productivity, i.e. winter, detritus will be an important sink for inorganic N removal. The high ^{15}N recovery in detritus was suprising since the original detritus had a high N content (23 mg g^{-1} dry tissue). Therefore, the detritus used in water with added $^{15}NO_3^-$-N probably accounted for even more ^{15}N immobilization due to a low initial N content (5 mg g^{-1} dry tissue).

Generally ^{15}N recovery in sediment was primarily organic N. Less than 1% of the added $^{15}NO_3^-$-N was recovered as sediment inorganic N. However, between 3 and 7% of the added $^{15}NH_4^+$-N was recovered as KCl extractable inorganic N in the sediment. The lower recovery of sediment inorganic ^{15}N in water with added $^{15}NO_3^-$-N was probably due to reduction to gaseous N via denitrification (Engler and Patrick, 1973). Some of the added ^{15}N was recovered as organic N in the water.

Plant uptake was the primary mechanism of N removal in water having water hyacinths. The ^{15}N unaccounted for was lost from the systems through a variety of possible transformations. A more thorough investigation would be required to establish the extent of algal N assimilation. Volatilization of NH_3-N in water without plant cover and denitrification in water with sediment are two possible mechanisms for N removal.

Conclusions

Generally as the rate of detritus addition increased, dissolved O_2 concentrations decreased in water with or without sediment and with or without plant cover. The decreasing dissolved O_2 concentrations were attributed to increasing heterotrophic respiration due to increasing amounts of C. Although this general relationship existed for all treatments, plant cover and sediment layer appeared to have more of a regulatory role in dissolved O_2 dynamics than detritus.

Water pH was constant in water having plant cover and sediment. The decreasing pH of water with plant cover and no sediment was attributed to NH_4^+-N assimilation by plants in exchange for H^+. The pH of open water was generally lower as the rate of detritus increased.

Detritus had no apparent effect on rate of N loss in water with water hyacinths. However, N loss was more rapid in open water as the rate of detritus increased.

Total plant $^{15}NH_4^+$-N uptake exceeded $^{15}NO_3^-$-N uptake. Both sediment and detritus appeared to be a potential N source for water hyacinths. Total ^{15}N recovered by water hyacinths ranged from 57 to 72% for added $^{15}NO_3^-$-N and 70 to 89% for added $^{15}NH_4^+$-N.

Less than 10% of the added $^{15}NH_4^+$-N was immobilized by detritus in water with plant cover. However, in water without plant cover, up to 35% of the added $^{15}NH_4^+$-N was associated with detritus. This suggests that during periods of low water hyacinth productivity, typical in cold weather conditions, detritus is an important sink for added N.

ANAEROBIC DIGESTION OF WATER HYACINTH

The potential productivity of water hyacinth has led to its selection as a biomass feedstock for methane generation while providing a means for treatment of nutrient-enriched waters Methane yields during anaerobic digestion depended on characteristics of the feedstock (Stack et al., 1978, Wolverton and McDonald, 1981) as well as digester operating conditions (Hashimoto et al , 1980) Sievers and Brune (1978) reported higher methane yields for digesters operating on swine waste as the C/N ratio increased. They concluded that the optimum C/N range for maximum methane production was 15 5/1 to 19/1 The optimum pH and temperature range for anaerobic digestion was 6 7 to 7 4 (Bryant, 1979) and 30 to 35°C (Mouse, 1981), respectively

Biogas and methane yields have been reported for water hyacinths using a variety of digesters Wolverton and McDonald (1981) reported methane yields of 0 07 to 0 20 L g^{-1} total solids (TS) for blended water hyacinths Hanisak et al (1980) found average methane yields of 0 24 L g^{-1} volatile solids (VS) from shredded water hyacinths in 162 L digesters at loading rates of 1 10 to 1 38 g VS L^{-1} day^{-1} and residence times of 30 to 38 days

Chynoweth et al (1983) reported methane yields of 0 19 and 0 28 L g^{-1} VS of water hyacinth and a 3 1 water hyacinth/primary sewage sludge blend, respectively, in 5 L daily-fed digesters with a loading rate of

1.6 g VS L^{-1} day^{-1}. Shiralipour and Smith (1984) reported average methane yields of 0.32 and 0.17 L g^{-1} VS water hyacinth shoot and root samples, respectively, in a bioassay test of 100 ml culture volume. They also concluded that the addition of N in growth media for water hyacinth production increased methane yields of both shoot and root samples.

Inoculum from operating anaerobic digesters is commonly added as a bacterial seed to initiate anaerobic digestion in new digesters (Sievers and Brune, 1978; Wolverton and McDonald, 1981; Field et al., 1984). Information on the effect of inoculum volume on gas production is limited.

The objectives of this study were 1) to determine C and N mineralization during anaerobic digestion of water hyacinth, 2) to determine the effect of inoculum volume on gas production, and 3) to evaluate effluent (solids and liquid) composition based on inoculum volume.

Materials and Methods

Water hyacinths, with either high or low tissue N content, were anaerobically digested at 35°C in 55 L batch digesters containing 2.5, 5, or 10 L of inoculum. Water hyacinths with a high N content (~34 g kg^{-1} dry wt plant tissue) were obtained from the wastewater treatment plant of the Reedy Creek Utility Company, Inc., at Walt Disney World near Orlando, Florida. Water hyacinths with a low N content (~10 g kg^{-1} dry wt plant tissue) were grown in nutrient-depleted water at Sanford, Florida. Both types of hyacinths were grown in ^{15}N labeled $(NH_4)_2SO_4$ for two weeks, frozen and chopped to 1.6 mm length using a Hobart Y 215 food processor.

The digesters received 4.7 kg fresh weight of the ^{15}N labeled water hyacinths and an inoculum volume of 2.5, 5 or 10 L. A control digester received 10 L of inoculum and no plant material. The inoculum used for plants with high N content was obtained from an operating continuous-fed upflow digester receiving a feedstock of water hyacinth and domestic sewage sludge in a blend ratio of 3:1 (Chynoweth et al., 1983). The inoculum used for the plants with low N content was obtained from a non-operating continuously-fed tank digester receiving water hyacinth as feedstock. Each digester was buffered with 210 g $NaHCO_3$ and tap water was used to bring each batch digester to 54.7 kg.

Gas production was monitored for 60 days. At the end of the digestion period, each digester was thoroughly mixed and the total contents were emptied into a 60 L tub. The digested materials were passed through a 1.00 mm fiberglass screen into a second 60 L tub to separate the digested biomass sludge from the effluent. The sludge was drained for 7 minutes and transferred into a polyethylene bag and placed directly into a freezer. The effluent was transferred to a water hyacinth production system.

The liquid samples from the digester effluents and screened effluents (sludge removed) were analyzed for pH, electrical conductivity (EC), total solids (TS), fixed solids (FS), volatile solids (VS) (APHA, 1980), total Kjeldahl N (TKN) (Nelson and Sommers, 1975), NH_4^+-N and NO_3^--N by steam distillation (Keeney and Nelson, 1982), and chemical oxygen demand (COD) (APHA, 1980). The screened effluent was also filtered through a 0.2 µm membrane filter and analyzed for Ca, K, Na and Mg by atomic absorption and P by an autoanalyzer.

The fresh plant material and digested sludge were freeze-dried (Thermovac-T) and analyzed for the following: TS, FS, VS, TKN (Nelson and Sommers, 1973), total carbon (TC) (LECO Induction Furnace 523-300), lignin, cellulose and hemicellulose (Goering and Van Soest, 1970), and ashed mineral constituents (Gaines and Mitchell, 1979).

Results and Discussion

Characteristics of Inocula

Characteristics of the two inocula varied considerably (Table 9). The inoculum used for plants with high N content (high N plants) contained higher levels of TS, VS, NH_4^+-N, TKN, and COD than the inoculum used for plants with low N content (low N plants). Characteristics of inoculum depended on the type of feedstock used for digestion (Stack et al., 1978) as well as digester operating conditions (Hashimoto et al., 1980). The inoculum used for high and low N plants came from digesters with feedstocks of a 3:1 water hyacinth/domestic sewage sludge blend and water hyacinths, respectively. Ammonium accounted for 68 and 92% of the total N of the inoculum from the water hyacinth/sewage sludge and water hyacinth feedstocks, respectively. Ammonium was the primary N source for methanogenic bacteria (Zeikus, 1977).

Carbon and Nitrogen Mineralization During Digestion

Biogas (CH_4 and CO_2) production, corrected to standard conditions (0°C and 0.1 MPa), is given in Table 10. Gas production essentially ceased after 60 days of digestion. Cumulative biogas production at 60 days for high N plants was approximately 21% less for 2.5 L of inoculum compared to 10 L. Furthermore, cumulative biogas production at 15 days

Table 9 Characteristics of the inocula used in the [illegible]

Inocula characteristics[†]							
NH_4^+ N	NO_3 N	TKN	COD	pH	TS	FS	VS
-- --mg L^{-1}		--------			--%	[illegible]	
		High N plant material					
1072	48	1530	14200	6.3	1.75	35.5	[illegible]
		Low N plant material					
535	21	562	784	7.7	0.29	83.1	16.9

[†] COD = Chemical oxygen demand, TS, FS, and VS = Total, fixed and volatile solids, respectively.

Table 18. Gas production during anaerobic digestion of high and low N water hyacinth plants.

Inoculum volume	Cumulative biogas production 15 days	 30 days	 60 days	Total Gas Yields biogas	 methane
--L--	-------	Liters-------	-------	---L g-1 VS---	
		High N plant material			
2.5	16.4	40.8	60.3	0.21	0.14
5	28.1	53.5	73.0	0.23	0.15
10	34.5	59.1	75.4	0.20	0.13
		Low N plant material			
2.5	16.9	45.5	67.9	0.25	0.16
5	20.0	51.6	72.6	0.27	0.17
10	14.7	52.2	67.4	0.25	0.16

was over twice as great for the digester receiving the largest amount of inoculum. However, for low N plants, the amount of inoculum did not appreciably effect cumulative biogas production during digestion.

Cumulative biogas production at 60 days was similar for both high and low N plants. Biogas production at 15 days was generally greater for high N plants. It appeared that N was not a limiting factor for total gas production in either digestion test.

Converting 60 day biogas production to biogas or methane yields (L g^{-1} VS added) is also presented in Table 10. Volatile solids included inputs from water hyacinths and inoculum. The average methane content of the biogas was 63.7 ± 5.2 % based on 18 samples. Surprisingly, biogas and methane yields were higher for the low N plants. This was caused by an increase of VS from inoculum used in digesters for high N plants. The inoculum used for high N plants contained 1.75% TS (66.5% VS of TS) (Table 9). The inoculum for low N plants contained 0.29% TS (16.9% VS of TS). Gas production expressed in these units suggested that inoculum volume did not appreciably effect total biogas or methane yields.

The average methane yields were 0.14 and 0.16 L g^{-1} VS added for high and low N water hyacinth plants, respectively. The methane yields were lower than those reported for continuously-fed digesters (Hanisak et al., 1980; Chynoweth et al., 1983). Batch digestion (once fed and sealed) would not promote maximum gas yields as frequent addition of fresh substrate enhances gas production (Price and Cheremisinoff, 1981).

Shiralipour and Smith (1984) reported that methane production for water hyacinth roots was lower than for shoots and that increasing N in water hyacinth growth media increased methane yields. Water hyacinths typically produced longer roots as water fertility declined (see p. 32).

Shoot root dry weight ratios of water hyacinth were higher when nutrients were not limiting and decreased significantly when plants grew in nutrient-poor waters (Reddy, 1984) It was assumed in the present study that gas production, both cumulative and yields, would be greater for the high N plants.

A mass balance of N is presented in Table 11. The organic N content decreased after anaerobic digestion for each treatment Mineralization of organic N to NH_4^+-N was the primary N transformation occurring during digestion The total N recovered was lower for low N water hyacinth plants The majority of the N was recovered in the effluent as NH_4^+-N. Most of the N placed in digesters was recovered in the effluent, although the proportion of NH_4^+ N of the total N tended to increase (Hashimoto et al , 1980; Field et al , 1984).

Approximately 30% of the organic N placed in the digesters was recovered as organic N in the digested sludge for both high and low N plants The organic N recovered in the screened effluent was 15 and 36% of the added organic N for high and low N plants, respectively The total organic N recovered as effluent or sludge organic N was 45 and 66% of added organic N for high and low N plants, respectively Therefore, a high N content of water hyacinth resulted in more mineralization of added organic N

Total ^{15}N recovered as $^{15}NH_4^+$-N in the screened effluent was 72 ± 4% for high N plants compared to 35 ± 9% for low N plants (Table 12) The organic ^{15}N recovered in digested sludge accounted for 20 ± 5% of the added ^{15}N from fresh water hyacinth plants regardless of N content Approximately 11 and 20% of the added ^{15}N was recovered as organic N in the screened effluent for digested high and low N plants, respectively

Table 11 Nitrogen balance for the batch digesters

	High N plant material			Low N plant material		
	2.5 L[†]	5 L	10 L	2.5 L	5 L	10 L
			%			
Nitrogen added						
Water hyacinth						
Organic N	10.39	10.39	10.39	3.24	3.24	3.24
Inoculum						
Organic N	1.15	2.31	4.61	0.07	0.14	0.27
Inorganic N	2.68	5.36	10.72	1.34	2.68	5.35
Total						
Organic N	11.54	12.69	14.99	3.31	3.37	3.51
Inorganic N	2.68	5.36	10.72	1.34	2.68	5.35
	14.22	18.05	25.72	4.64	6.05	8.86
Nitrogen recovered						
Screened effluent						
Organic N	1.48	2.41	2.02	1.26	1.31	1.09
Inorganic N	8.81	11.60	15.81	1.20	2.63	4.98
Digested sludge						
Organic N	2.86	4.91	4.94	1.17	0.85	0.87
Total						
Organic N	4.34	7.32	6.96	2.43	2.16	1.96
Inorganic N	8.81	11.60	15.81	1.20	2.63	4.98
	13.15	18.91	22.77	3.63	4.79	6.94
% Recovered	92	105	89	78	79	78

[†] Volume (liters) of inoculum

Table 12. Nitrogen-15 balance for the batch digesters

	High N plant material			Low N plant material		
	2.5 L[†]	5 L	10 L	2.5 L	5 L	10 L
	------	-------	g	-------	------	------
^{15}N Added						
Water hyacinth						
Organic N	10.39	10.39	10.39	3.24	3.24	3.24
^{15}N Recovered						
Screened effluent						
Organic N	1.18	1.48	0.93	0.84	0.65	0.34
Inorganic N	7.06	7.78	7.68	0.79	1.25	1.34
Digested Sludge						
Organic N	1.67	2.53	2.21	0.87	0.57	0.52
Total	9.91	11.79	10.82	2.50	2.46	2.20
% Recovered	95	113	104	77	76	68

[†] Volume (liters) of inoculum

The low total recovery of N and ^{15}N for low N plants after anaerobic digestion is difficult to explain. Nitrogen cycling during anaerobic digestion was primarily mineralization of organic N or immobilization of inorganic N. Volatilization of NH_3-N may occur but the potential increases as NH_4^+-N concentrations increase or at higher pH values (Freney et al., 1983). Each digester received 210 g $NaHCO_3$ as a buffer and the pH after digestion was similar for all digester effluents. The NH_4^+-N concentrations after digestion were much higher for the high N plants (Table 13).

Effluent composition

Characteristics of the digester effluents prior to sludge separation are presented in Table 13. Generally, as the rate of inoculum increased, there were increases in EC, NH_4^+-N, TKN, and TS. The COD increased with increasing inoculum volume for digesters with high N plants.

Characteristics of the screened effluent (sludge removed) are reported in Table 14. Removing the digested biomass sludge from the digester effluents decreased the EC, NH_4^+-N and TKN. The screened effluent from the low N plants contained more Ca, Mg, K, and Na, and less P than the screened effluent from the high N plants.

Characteristics of the fresh plant biomass and digested biomass sludge are given in Table 15. Anaerobic digestion resulted in increases in TC and TKN of sludge compared to fresh plant biomass. The increases in sludge TKN after digestion of low N plants caused a reduction of the C/N ratio from 35 to 16. The changes in TC or TKN of the digested high N plants did not appreciably alter the C/N ratio. The digested sludge

Table 13 Characteristics of digester effluents before sludge removal.

Inoculum volume	pH	EC	COD	NH_4^+-N	TKN	TS	VS	FS
-mL-		dS		mg L^{-1}------		%	-% of TS-	
				High N plant material				
2.5	7.4	4.5	3030	186	238	0.488	40.3	59.7
5	7.6	5.1	4280	210	315	0.570	38.7	61.3
10	7.4	5.8	5050	294	406	0.610	38.9	61.1
Control	7.7	NA[‡]	4170	205	258	0.535	36.6	63.4
				Low N plant material				
2.5	7.3	8.8	1880	50	70	0.445	28.9	71.1
5	7.2	8.8	2340	70	112	NA	37.3	62.7
10	7.3	7.8	1840	112	154	0.488	28.8	71.2
Control	7.8	NA	108	88	120	0.315	14.0	86.0

[†] COD = Chemical oxygen demand, TS, FS and VS = Total, fixed and volatile solids, respectively
[‡] NA = Not available.

Table 14 Characteristics of screened effluents (sludge removed) after digestion

Inoculum volume	Screened Effluent Characteristics†										
	pH	EC	NH_4-N	TKN	Ca	Mg	K	Na	P	TS	VS	FS
-L-		dS	----------------	-------	mg L^{-1}	-------	-------	-------	-------	%	% of TS	
					High N plant material							
2.5	7.6	4.3	161	188	32	13	82	1140	12.8	0.357	32.4	67.6
5	7.6	4.7	212	256	30	12	80	1160	12.8	0.342	41.5	58.5
10	7.6	5.3	289	326	23	14	123	1160	11.5	0.369	41.0	59.0
					Low N plant material							
2.5	7.5	5.6	22	45	147	50	195	1220	3.1	0.367	30.3	69.7
5	7.4	5.9	48	72	53	53	245	1240	4.6	0.408	30.5	69.5
10	7.5	6.7	91	111	61	63	325	1200	4.5	0.425	28.1	71.9

†TS, VS, FS = Total, volatile and fixed solids, respectively

Table 15 Characteristics of fresh and digested biomass residues.

Biomass residue	Inoculum volume	VS	FS	TC	TKN	C/N	Lig[†]	Cell	Hemc	Ca	K	Mg	Na	Fe	[illegible]
	--L--	-	$g\ kg^{-1}$------				---------			$g\ kg^{-1}$					
							High N plant material								
Fresh		834	188	385	34.0	12	43	187	182	17.8	23.5	3.2	8.0	1.80	[illegible]
Digested	2.5	843	157	449	37.2	12	136	159	NA[‡]	17.8	2.2	1.8	15.3	4.04	[illegible]
Digested	5	827	173	446	39.3	12	130	207	180	18.4	3.8	2.2	28.0	4.78	0.92
Digested	10	848	154	441	30.8	14	111	168	NA	17.8	3.8	2.0	20.8	3.88	1.00
							Low N plant material								
Fresh		839	181	373	10.8	35	83	288	247	21.0	22.0	6.7	10.9	1.88	[illegible]
Digested	2.5	879	121	441	27.4	18	149	177	NA	22.1	2.8	2.8	14.8	5.72	[illegible]
Digested	5	888	134	425	28.8	18	145	181	242	24.2	2.8	2.2	17.4	7.16	2.04
Digested	10	847	153	433	25.2	17	183	163	NA	28.8	4.6	2.7	19.5	8.40	2.18

[†] Lig, Cell, Hemc = Lignin, cellulose and hemicellulose

[‡] NA = Not available

had a higher lignin content compared to fresh plant biomass. The increase in lignin was due to the loss of readily decomposable C during anaerobic digestion. Lignin appears to be practically inert to anaerobic digestion (Hashimoto et al., 1980). Generally, there was a decrease in cellulose after digestion. The hemicellulose remained similar for the fresh plant biomass and digested sludge. Anaerobic digestion resulted in losses of K and Mg from fresh plant biomass, but increased the concentration of sludge Ca, Na, Fe, and Zn.

Conclusions

Cumulative biogas production at 60 days was similar for high (~34 g N kg^{-1} dry wt tissue) and low (~10 g N kg^{-1}) N plants suggesting that long term digestion of water hyacinth was not influenced by initial N content. Effects of inoculum volume on cumulative biogas production were seen at 15 days for high N plants but not low N plants. Conversion of cumulative biogas production into biogas and methane yields (L g^{-1} VS added) showed that low N plants produced more biogas and methane than high N plants. This was due to increase of TS (and consequently VS) in digesters of high N plants from the inoculum source, since cumulative gas production was similar for both types of plants.

Mineralization of organic ^{15}N to $^{15}NH_4^+$-N accounted for 72 and 35% of added ^{15}N for high and low N plants, respectively. Approximately 20% of the added ^{15}N was recovered as organic N in sludge for both types of plants. A low ^{15}N recovery was observed for low N plants.

Increasing inoculum volume increased electrical conductivity, NH_4^+-N, TKN, and TS of the digester effluents. The digested biomass sludge had higher levels of TC, TKN, lignin, Ca, Na, Fe, and Zn, and lower levels of K and Mg compared to the fresh plant biomass. The C/N ratio of the fresh plant biomass with a low tissue N content decreased from 35 to 16 after digestion. The C/N ratio of the fresh plant biomass with a high tissue N content was the same as the digested biomass sludge (C/N=12).

TREATMENT OF ANAEROBIC DIGESTER EFFLUENTS USING WATER HYACINTHS

An integrated approach of wastewater renovation using aquatic macrophytes with utilization of biomass for energy production is economically appealing. The plant biomass produced in these systems, along with other wastes such as sewage sludge or animal waste could be anaerobically digested to produce methane (Stack et al., 1981; Shiralipour and Smith, 1984). This process generates a waste by product which must be disposed of, or preferably utilized to reduce the cost of energy production, in an environmentally-safe manner. The waste by-product consists of digested sludge and a large volume of effluent. Integrating wastewater renovation through water hyacinth production provides an internal option for the disposal of effluent generated during conversion of biomass into methane.

The effluent composition of anaerobic digesters varied with type of feedstock used in digestion (Stack et al., 1981). Information on chemical composition of effluents from sewage or animal wastes was readily available (Sommers, 1977, Field et al., 1984). However, anaerobic digestion of plant biomass has only recently gained attention in the United States and information on composition or disposal of the effluent was limited (Hanisak et al., 1980, Atalay and Blanchar, 1984).

Digester effluents have high concentrations of BOD, NH_4^+-N, K and Na (Atalay and Blanchar, 1984; Field et al., 1984), while the divalent cations and metals were concentrated in the sludge (Sommers, 1977, Field et al., 1984).

Water hyacinth based wastewater treatment systems have already been evaluated for use in treating primary and secondary sewage effluents (Wolverton and McDonald, 1979; Reddy et al., 1985) and anaerobic digester effluent (Hanisak et al., 1980). The potential productivity of water hyacinth in nutrient-enriched waters has led to its selection in alternative methods of wastewater renovation, particularly in areas where growth is not restricted by climatic limitations.

Use of water hyacinth for digester effluent treatment is particularly attractive, because of its ability to grow in waters with high elemental concentrations. The biomass produced could be returned to the digester as a feedstock for methane production. Hanisak et al. (1980) determined that 64.5% of (liquid and sludge) N in diluted effluents from anaerobically digested water hyacinth could be reassimilated by water hyacinths. Diluting the effluent does not address the full potential of water hyacinth to grow under these nutrient and salt enriched conditions. Haller et al. (1974) concluded that water hyacinth will not live in waters containing sustained salt concentrations in excess of 2500 mg L^{-1}. Optimal dilution of these concentrated effluents to obtain maximum water hyacinth yields and nutrient removal was not reported.

The objectives of this study were to 1) evaluate water hyacinth productivity in anaerobic digester effluents obtained from digesters receiving different types of water hyacinth as feedstock, and, 2) determine ^{15}N recovery by water hyacinth growing in digester effluents from digested ^{15}N labeled water hyacinth biomass.

Materials and Methods

Anaerobic digester effluents were obtained from six 55 L batch digesters containing water hyacinth with a high or low tissue N content as feedstock. Water hyacinths with low (~10 g N kg^{-1} dry plant tissue) and high (~34 g N kg^{-1}) tissue N content were grown in nutrient-depleted water and sewage effluent, respectively. After removal from their respective growth media, the hyacinths were grown in ^{15}N labeled $(NH_4)_2SO_4$ nutrient solution for two weeks, frozen, and chopped to 1-6 mm length using a Hobart T 215 feed processor.

The water hyacinths were anaerobically digested for four months in 55 L batch digesters. Each digester received 4.7 kg (fresh weight) of the ^{15}N labeled water hyacinth, 2.5, 5, or 10 L volume of inoculum from anaerobic digesters receiving water hyacinth as feedstock, and were buffered with 210 g $NaHCO_3$. After digestion, the biomass sludge was separated from the effluent by passing the total contents of the digesters through a 1.00 mm fiberglass screen.

The screened effluents (sludge removed) were analyzed for total solids (TS), volatile solids (VS), fixed solids (FS) (APHA, 1980), total Kjeldahl N (TKN) (Nelson and Sommers, 1975), NH_4^+-N and NO_3^--N by steam distillation (Keeney and Nelson, 1982), electrical conductivity (EC) (Hach Mini Conductivity Meter) and pH (Orion Model 404 Specific Ion Meter). Samples passed through a 0.2 μm membrane filter were analyzed for Na, K, Mg and Ca by atomic absorption and ortho P colorimetrically after reacting with ammonium molybdate.

Six water hyacinth plants were placed in 10 L of undiluted or diluted effluents in containers having a surface area of 0.051 m^2. The water hyacinth plants were collected from the University of Florida's

Givens Air research reservoirs in Gainesville, Florida. The plants were clipped of dead tissue and acclimated to greenhouse conditions for two weeks prior to treatments. The studies to evaluate the potential of water hyacinth to treat digester effluents were conducted for a period of 22 days in March and September, 1984. The daily maximum greenhouse temperatures ranged from 19 to 37°C in March and 27 to 36°C in September.

There were a total of 10 treatments, replicated three times, as described below. Treatments 1 to 3 were diluted effluents from digesters containing high N water hyacinths and inoculum volumes of 2.5, 5, and 10 L, respectively. The dilutions were 1:8, 1:4, and 1:3 effluent : tap water for digester effluents with inoculum volumes of 2.5, 5, and 10 L, respectively. Treatments 4 to 6 were undiluted effluents from digesters containing high N plants and inoculum volumes of 2.5, 5, and 10 L, respectively. Treatments 7 to 9 were undiluted effluents from digesters containing low N plants and inoculum volumes of 2.5, 5, and 10 L, respectively. The final treatment was a modified 10% Hoagland's solution (p. 42) to serve as a control. Characteristics of the inoculum and effluents are given in the Anaerobic Digestion of Water Hyacinth chapter (pp. 50 to 75).

Plant samples collected initially and at the conclusion of the experiment were analyzed for dry weights, TKN (Nelson and Sommers, 1973), Na, K, Ca and Mg by atomic absorption and P by an autoanalyzer. Water samples were collected at 0, 1, 2, 3, 4, 8, 15, and 22 days and analyzed for NH_4^+-N and NO_3^--N by steam distillation (Keeney and Nelson, 1982), TKN (Nelson and Sommers, 1975), Ca, Mg, K, Na by atomic absorption and P by an autoanalyzer. Electrical conductivity (Mach Mini

Conductivity Meter), pH (Orion Model 404 Specific Ion Meter) and dissolved O_2 (Yellow Springs Instrument Model 54 O_2 Meter) were measured every other day. The ^{15}N analyses of plant and water samples were conducted on a Micro Mass 602 spectrometer.

Results and Discussion

Chemical Composition of the Effluents

Characteristics of digester effluents used in the study are given in Table 16. The initial pH of the effluent sources and nutrient medium were similar. The digester effluents had a wide range of EC, NH_4^+-N, TKN and other nutrients. This provided an opportunity to evaluate water hyacinth growth under diverse media conditions. The EC of the nutrient medium and diluted effluents ranged from 0.7 to 2.3 dS m^{-1}. The EC of the undiluted effluents ranged from 4.3 to 6.7 dS m^{-1}.

The NH_4^+-N and TKN concentrations of the undiluted effluents from digested plants with a high N content (high N plants) were greater than those of the undiluted effluents of digested plants with a low N content (low N plants). The NH_4^+-N concentrations ranged from 23 to 104 mg L^{-1} for diluted effluents and 24 to 289 mg L^{-1} for undiluted effluents, respectively.

High Na and K concentrations were noted for undiluted effluents. The highest levels of P were in undiluted effluents of digested high N plants and the highest levels of Ca and Mg were in undiluted effluents of digested low N plants. The critical levels of Na, K, Ca and Mg needed to achieve maximum water hyacinth yields are relatively unknown. The NH_4^+-N, K and Mg concentrations were higher than levels reported for water hyacinths cultured in primary or secondary sewage effluent (Reddy et al., 1985).

Table 16 Initial characteristics of the digester effluents and nutrient medium

Inoculum volume	pH	EC	NH_4^+-N	TKN	Na	K	P	Ca	Mg
--L--		dS	------------------------	mg L^{-1}	------				
				Diluted effluents from high N plants					
2.5	7.8	0.7	23	26	166	20	2.8	[illegible]	[illegible]
5	7.7	1.6	65	72	360	44	5.5	[illegible]	[illegible]
10	7.7	2.3	104	124	450	62	4.7	[illegible]	[illegible]
				Undiluted effluents from high N plants					
2.5	7.6	4.3	161	188	1140	82	12.8	32	[illegible]
5	7.6	4.7	212	256	1160	80	12.8	30	[illegible]
10	7.6	5.3	289	326	1160	123	11.5	23	[illegible]
				Undiluted effluents from low N plants					
2.5	7.5	5.6	24	45	1220	195	3.1	147	50
5	7.4	5.8	49	72	1240	245	4.6	53	53
10	7.5	6.7	87	111	1200	325	4.5	61	63
				Nutrient medium					
	7.5	0.7	20	21	11	24	[illegible]	[illegible]	[illegible]

Productivity of Water Hyacinths

Total dry weight gains of water hyacinths were consistently less in the undiluted effluents (Fig 15) Complete death of plants was observed in four undiluted effluents. The loss of dry weight for these treatments was probably due to leaching of soluble plant constituents after plant death

The highest dry weight gains were associated with the lowest EC However, plants survived in undiluted effluents having EC levels of 5.6 and 5.9 dS m^{-1} (5600 and 5900 µmhos cm^{-1}) These EC levels were equivalent to -2900 and 3200 mg NaCl L^{-1} and were higher salt concentrations reported for water hyacinth survival (Penfound and Earle, 1948, Haller et al , 1974) The undiluted effluents had Na levels in excess of 1100 mg L^{-1} Apparently water hyacinth has a wide range of adaptability to media composition and, therefore, total salt concentration is not a good criterion for determining plant survival

The diluted effluents were excellent media for plant growth and the gains in dry weight were consistently higher compared to the nutrient medium (Fig. 15). The highest dry weight gain was in the diluted effluent having N and P concentrations of 65 and 5 5 mg L^{-1}, respectively, and a N/P ratio of 11.8:1 Sato and Kondo (1981) reported maximum yields of water hyacinth at a N and P concentration of 50 and 13 8 mg L^{-1}, respectively, and a N/P ratio of 3.7:1. Dry weight gains were noted for two of the undiluted effluents However, tissue damage was noted in all undiluted effluents

Tissue damage in undiluted effluents was observed within 24 hr after study initiation Two types of leaf tissue damage were observed Damaged leaves on younger shoots had burnt (brown) tips which curled up

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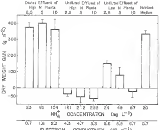

Figure 15 Dry weight gains of water hyacinths [illegible] and nutrient medium

towards the center of the plant. The other leaf tissue damage on young and older leaves was curling of the entire leaf towards the center of the leaf. Stem damage in undiluted effluents was noticeable after 2 days. Damaged stems collapsed under slight manual pressure. The destruction of chlorophyll in stems and leaves was widely observed in the undiluted effluents.

The extent and spread of tissue damage increased in undiluted effluents with increasing EC and NH_4^+-N concentrations. At the end of one week, all plants in four undiluted effluents were dead. The EC and NH_4^+-N concentrations of these effluents ranged from 4.3 to 6.7 dS m^{-1} and 67 to 289 mg N L^{-1}, respectively. The shoots began to separate from the roots at the water surface and the submerged roots sank. Root separation also accounted for the negative dry weight gains of plants in undiluted effluents.

Three treatments showed occasional visual signs of tissue damage, i.e., the nutrient medium and 2 diluted effluents. Visual signs of plant damage but noticeable gains in plant dry weights were observed in the diluted effluent having an initial NH_4^+-N concentration of 104 mg L^{-1} and two undiluted effluents having NH_4^+-N concentrations of 24 and 49 mg L^{-1}. All remaining treatments resulted in plant death, apparently due to high EC or high NH_4^+-N concentrations, or a combination of both.

Although water hyacinth plants struggled to survive in the undiluted effluents, algal activity was noted in all undiluted effluent treatments. Upon emptying the containers, algae were found attached to the side and bottom surfaces of the plastic containers.

Nitrogen Removal

First-order kinetic equations were used to described NH_4^+-N loss from the effluents. An integrated rate equation for a first-order reaction is expressed as:

$$\ln C_o / C_t = kt$$

where C_o = initial NH_4^+-N concentration in the effluent,

C_t = final concentration at time = t,

k = first order rate constant ($days^{-1}$)

The rate constant is calculated by solving for k:

$$k = \ln C_o / C_t * 1/t$$

The rate constants for diluted effluents ranged from 0.228 to 0.593 day^{-1} (Table 17). The rate constants for undiluted effluents ranged from 0.175 to 0.446 day^{-1}.

The time required for a 50% reduction in initial NH_4^+-N concentration generally increased with decreasing dry weight gains and increasing NH_4^+-N concentrations. The shortest reduction times were associated with rapid plant growth (50% reduction in 1.12 to 3.04 days). Plant assimilation was probably the primary mechanism of NH_4^+-N loss in these treatments. Reddy (1983) reported a 50% reduction of inorganic N from agricultural drainage water in 18 days. The time required for a 50% reduction of NH_4^+-N in effluents resulting in plant death was 1.98 to 3.96 days. Mechanisms of NH_4^+-N loss in treatments resulting in plant death included microbial assimilation and NH_3-N volatilization. The pH of the effluents ranged from 7.0 to 7.9 in treatments with actively growing plants but increased from 8.2 to 9.3 in treatments where plant death was observed and algal growth increased (APPENDIX A, Table 27). The potential of NH_3-N volatilization increases as NH_4^+-N concentrations increase or at higher pH values (Freney et al., 1983).

Table 11. First-order kinetic coefficients of NH_4^+-N loss with time.

Inoculum volume	Initial NH_4^+-N conc	k	Reduction time	R^2
--L--	mg L^{-1}	day^{-1}	days	
		Diluted effluents from high N plants		
2.5	23	0.593	1.12	0.722
5	65	0.449	1.54	0.917
10	104	0.228	3.04	0.951
		Undiluted effluents from high N plants		
2.5	161	0.232	2.98	0.938
5	212	0.207	3.35	0.947
10	289	0.175	3.96	0.982
		Undiluted effluents from low N plants		
2.5	24	0.446	1.55	0.850
5	49	0.325	2.13	0.885
10	87	0.350	1.98	0.845
		Nutrient medium		
	20	0.281	2.47	0.973

[†] Time required for 50% reduction in initial NH_4^+-N concentration.

Clock (1968) reported a 75% reduction of NO_3^--N in 5 days [illegible] hyacinths growing in secondary sewage effluent. A 75% reduction of NH_4^+-N in digester effluents required 2.3 to 6 days for treatments where positive dry weight gains were observed. For treatments resulting in plant death, 4 to 8 days were required to remove 75% of the NH_4^+-N.

Nitrogen-15 plant assimilation was observed for all treatments although a low recovery was observed in treatments resulting in plant death (2 to 16%) (Table 18). The ^{15}N recovery by plants for the other treatments ranged from 36 to 77%. The majority of the ^{15}N was found in the shoot material for all treatments (54 to 73%). Approximately 75% of the ^{15}N was unaccounted for in treatments resulting in plant death. Microbial assimilation and NH_3-N volatilization were probably important NH_4^+-N removal processes in undiluted effluents where plant death was observed.

Plant Tissue Chemical Composition

Plants survived in the diluted effluents but death was noted for plants in the undiluted effluents from digested high N plants. Mineral constituents from plants growing in these effluents were analysed to isolate individual cation and P assimilation or loss from living and dead plant tissue.

The concentrations of plant tissue (root and shoot fractions) Na, K, P, Ca and Mg are reported in Table 19. The original plant tissue had low concentrations of Na and P in both shoot and root material, but higher concentrations of K, Ca and Mg in the shoots compared to the roots. There were large increases in Na for both shoots and roots of the surviving and dead plants. The root K concentrations increased for surviving plants but decreased for dead plants. The P concentrations

Table 16. Nitrogen-15 [illegible] effluents

Inoculum volume	Available 15N	Recovered by plants		^{15}N Recovery		
		Roots	Shoots	Plants	Water	Unaccounted
L	------------mg------	-----		------% of applied ^{15}N--		
Diluted effluents from high N plants						
2.5	225	44	103	66	7	27
5	649	80	199	43	9	48
10	1044	101	272	36	3	61
Undiluted effluents from high N plants						
2.5	1610	20	45	4	19	77
5	2120	18	41	3	27	40
10	2890	19	46	2	25	74
Undiluted effluents from low N plants						
2.5	236	62	119	77	6	17
5	487	71	124	40	4	56
10	869	63	73	16	6	78

Table 19 Distribution of nutrients in water hyacinth shoots and roots in diluted or undiluted effluents of digested high N plants

Inoculum volume	Na	K	P	Ca	Mg
L			g kg^{-1}		
			SHOOTS		
		Diluted effluents from high N plants			
2.5	13.3	13.3	2.4	16.9	9.2
5	17.5	19.3	3.9	17.8	9.1
10	18.0	23.2	5.0	15.6	8.6
		Undiluted effluents from high N plants			
2.5	21.0	18.0	3.5	18.2	5.6
5	20.5	17.7	3.9	22.6	6.2
10	19.2	14.5	3.8	19.3	5.7
LSD (0.05)	4.4	7.1	1.3	3.7	1.9
		Original shoot tissue			
	2.8	22.0	2.7	15.4	5.7
			ROOTS		
		Diluted effluents from high N plants			
2.5	14.5	7.1	2.1	6.1	4.6
5	17.8	8.9	2.6	6.0	4.7
10	16.0	12.4	3.4	9.0	4.2
		Undiluted effluents from high N plants			
2.5	16.2	3.1	11.2	26.5	4.6
5	16.8	3.3	10.3	23.6	4.3
10	17.2	3.3	10.2	24.6	4.1
LSD (0.05)	3.0	3.7	1.9	3.0	0.7
		Original root tissue			
	4.6	5.5	2.8	6.6	2.6

increased for the dead plant root material but remained similar for the other materials. The Ca content increased for the shoots of all plants and the roots of the dead plant material. The Mg content increased for roots of all plants and shoots of the surviving plants.

The net assimilation or loss of plant nutrients from plants in diluted or undiluted effluents from digested high N plants are reported in Table 20. The plants in diluted effluents assimilated large amounts of Na and K, apparently because these nutrients move rapidly with the transpiration stream. The net shoot assimilation of all nutrients was greater than the net root assimilation.

The dead plants from undiluted effluents showed a net loss of K but net gains of the other nutrients. Potassium, Na, Ca and Mg were reported as being rapidly lost during the early leaching phase of plant decomposition in fresh water (Boyd, 1970b; Davis and van der Valk, 1978). Most of the Na moved into the shoot region. Generally the net gains of P and Ca were found in dead plant roots compared to shoots of surviving plants in diluted effluents.

Final Chemical Composition of the Effluents

Characteristics of the digester effluents and nutrient medium after water hyacinth treatment are given in Table 21. For treatments where plant dry weight gains were observed, generally there was large reductions of the elements analyzed in the effluents. For the treatments resulting in plant death, K and Mg increased and the reductions of other elements were of lesser magnitude.

The largest reductions of EC (49 ± 7% reduction), K (93 ± 3%) and P (92 ± 3%) were observed in diluted effluents. The highest gains of

Table 20 Net assimilation or loss of plant nutrients in diluted or undiluted effluents from digested high N plants

Inoculum volume	Na	K	P	Ca	Mg
--L--			------mg m^{-2} day^{-1}--------		
			SHOOTS		
		Diluted effluents from high N plants			
2.5	240	35	19	179	126
5	353	197	57	223	139
10	333	220	69	148	110
		Undiluted effluents from high N plants			
2.5	190	-23	10	42	4
5	201	-36	15	89	9
10	181	-92	11	35	-4
LSD (0.05)	64	101	17	62	27
			ROOTS		
		Diluted effluents from high N plants			
2.5	217	80	14	53	59
5	273	111	22	50	61
10	227	155	32	94	48
		Undiluted effluents from high N plants			
2.5	40	-26	31	73	2
5	50	-29	32	72	1
10	70	-29	42	103	3
LSD (0.05)	57	50	27	60	14

Table 21. Characteristics of the digester effluents and nutrient medium after water hyacinth treatment.

Inoculum volume	pH	EC	NH_4^+ N	TKN	Na	K	P	Ca	Mg
--L--		dS	--------------------mg L^{-1}---------						
			Diluted effluents from high N plants						
2.5	7.2	0.4	< 1	4.3	80	2	0.3	14	[illegible]
5	7.5	0.7	< 1	6.0	173	2	0.4	16	[illegible]
10	7.5	1.2	< 1	3.8	297	4	0.3	19	[illegible]
			Undiluted effluents from high N plants						
2.5	8.7	4.2	22.2	39.4	873	103	10.5	[illegible]	[illegible]
5	8.6	4.4	31.9	80.2	903	134	[illegible]	[illegible]	[illegible]
10	8.4	4.9	49.9	87.9	950	163	[illegible]	[illegible]	[illegible]
			Undiluted effluents from low N plants						
2.5	8.8	4.2	< 1	4.3	450	72	1.2	23	24
5	9.2	4.6	< 1	5.1	523	98	1.2	10	33
10	9.3	4.8	< 1	8.6	547	159	1.3	10	38
			Nutrient Medium						
	3.5	0.7	< 1	1.8	10	1	0.5	42	17

plant dry weight were also noted for these effluents. Ammonium was 1.0 mg L^{-1} and TKN was reduced 83 to 97% in these effluents after 22 days. Sodium reduction was in the range of 34 to 52%. Calcium and Mg decreased for the 2.5 and 5 L inoculum diluted effluents but increased for the 10 L inoculum diluted effluent.

Intermediate reductions of EC (25 ± 3%), K (58 ± 6%) and P (69 ± 7%) were observed in undiluted effluents from digested low N plants. Ammonium was < 1.0 mg L^{-1} and TKN was reduced 90 to 93% in these effluents after 22 days. Sodium decreased 54 to 63% after 22 days. The largest reductions of Ca (82 ± 4%) and Mg (43 ± 8%) were found in these effluents.

Plant death was noted in all undiluted effluents from digested high N plants. These treatments had the lowest reductions of EC (5 ± 3%) and P (26 ± 10%). Plants were removed after 10 days following complete death of aerial tissue and separation of root masses. The death of plant tissue resulted in an increase of K and Mg in the effluents. Sodium was reduced 18 to 23%. Calcium reduction was in the range of 52 to 70%. The loss of NH_4^+-N (83 to 98% reduction) was probably due to NH_3 volatilization and microbial assimilation.

Conclusions

The highest dry weight gains were for plants growing in diluted effluents with EC levels ranging from 0.7 to 2.3 dS m^{-1} and NH_4^+-N concentrations of 23 to 104 mg L^{-1}. Plants survived and grew in two undiluted effluents with EC levels of 5.6 and 5.9 dS m^{-1} and NH_4^+-N concentrations of 24 and 40 mg L^{-1}. All other EC and NH_4^+-N combinations of undiluted effluents resulted in plant death.

A first-order kinetic equation was used to describe NH_4^+-N loss with time. Rate constants for diluted effluents ranged from 0.228 to 0.593 day^{-1}. Rate constants for undiluted effluents ranged from 0.175 to 0.448 day^{-1}. The time required for a 50% reduction of NH_4^+-N was 1.12 to 3.04 days for treatments with positive water hyacinth dry weight gains. A 50% reduction of NH_4^+-N in treatments resulting in plant death required 1.98 to 3.98 days. Plant assimilation was one of the primary mechanisms of NH_4^+-N loss in the systems with actively growing plants. Microbial assimilation and NH_3-N volatilization were probably important mechanisms of NH_4^+-N removal for treatments resulting in plant death.

Plant assimilation accounted for a 36 to 77% recovery of effluent ^{15}N for surviving plants. Only 2 to 16% of the ^{15}N was recovered in dead plant tissue. Approximately 75% of the ^{15}N was unaccounted for in effluents resulting in plant death.

Surviving plants assimilated large amounts of Na, K, Ca and Mg while dead plants lost K and had small gains of Ca and P. Sodium accumulated in dead plant tissue. Death was attributed to an indiscriminate salt injury and/or NH_3-N toxicity.

The largest reductions of EC, K and P were observed in diluted effluents. The highest plant dry weight gains were also found in these effluents. Potassium and Mg increased in effluents where plant death was noted.

DECOMPOSITION OF FRESH AND ANAEROBICALLY DIGESTED PLANT BIOMASS IN SOIL

Anaerobic digestion of plant biomass, sewage sludge, or animal wastes generates a waste by-product which must be disposed of, or preferably utilized, in an environmentally-safe manner. Disposal of the digested sludge by land application is one option often considered (Miller, 1974, Terry et al., 1979, Atalay and Blanchar, 1984). The digested biomass sludge differs chemically from the fresh plant biomass. A consequence of anaerobic digestion is a reduction of the more readily decomposable C of the plant tissue during production of CH_4 and CO_2. Many sludges contained relatively large amounts of Ca, Mg, P and Zn and lower contents of soluble elements such as K (Sommers, 1977).

Miller (1974) concluded that anaerobically digested sewage sludge was rather resistant to further decomposition with a maximum of 20% of the added C was evolved as CO_2 during a 6-month incubation. Tester et al. (1977) reported 16% of added C from composted sewage sludge was evolved as CO_2 during 54 days of incubation. Hsieh et al. (1981a) showed that activated sludge had a much higher C mineralization rate compared to digested sludge due to a larger portion of active organic C.

Epstein et al. (1978) determined that the percentage of added N mineralized from digested sludge remained essentially constant irrespective of application rate. However, Ryan et al. (1973) and Stark and Clapp (1980) observed a reduced rate of N mineralization with increasing rate of sewage sludge application. Nitrogen mineralization potential was found to be 30 and 38% of organic N in activated and digested sludge, respectively, during 60 days of incubation (Hsieh et al., 1981b).

Much of the available information dealt with land application of anaerobically digested sewage sludge (Miller, 1974, Terry et al., 1979), and limited data was reported on the decomposition of sludge obtained from the anaerobic digestion of plant biomass (Atalay and Blanchar, 1984). The objective of this study was to evaluate the decomposition and N mineralization rates of anaerobically digested plant biomass added to soil. Four materials were evaluated: fresh plant biomass with a low or high tissue N content, and their respective anaerobically digested residues.

Materials and Methods

Surface (0-15 cm depth) soil samples of a Kendrick fine sand (Arenic paleudult) were collected at the Agronomy Farm, University of Florida in Gainesville, Florida. The soil was air-dried and passed through a 2 mm sieve. The soil had a particle size distribution of 92.9% sand, 4.6% silt, and 2.5% clay. The CEC was 3.44 cmol(+) kg^{-1} soil with a base saturation of 47%.

Water hyacinths with low (10 g N kg^{-1} dry plant tissue) and high (34 g N kg^{-1}) tissue N content were grown in nutrient depleted water and sewage effluent, respectively. After removal from their respective growth media, the hyacinths were grown in ^{15}N labeled $(NH_4)_2SO_4$ nutrient solution for two weeks, frozen, and chopped to 1.6 mm length using a Hobart T 215 food processor.

The water hyacinths were anaerobically digested for four months in 55 L batch digesters. Each digester received 4.7 kg (fresh weight) of the ^{15}N labeled water hyacinth, 5 L of an inoculum from anaerobic digesters receiving water hyacinth as feedstock, and were buffered with 210 g $NaHCO_3$. After digestion, the biomass sludge was separated from the effluent by passing the total contents of the digester through a 1.00 mm fiberglass screen.

Samples of the fresh water hyacinth and anaerobically digested water hyacinth sludge were freeze-dried (Thermovac T) and ground through a 0.84 mm screen of a Wiley Mill. The freeze-dried materials were characterized for lignin, cellulose and hemicellulose (Goering and Van Soest, 1970), ashed mineral constituents (Gaines and Mitchell, 1979), total solids (TS), volatile solids (VS), total C (TC) (LECO Induction Furnace 523-300), and total Kjeldahl N (TKN) (Nelson and Sommers, 1973). Carbon/nitrogen (C/N) ratios of the four residues were calculated from percentage TC and TKN.

Fifty gram soil samples were preincubated for 5 days at a water content adjusted to 0.01 MPa before addition of the residues. The freeze-dried materials were added to the soil at a rate of 5 g (dry wt) kg^{-1} soil (10 Mg ha^{-1}) and incubated for 90 days at 27°C. Water content was adjusted to 0.01 MPa every 15 days. Ambient laboratory air, with CO_2 and NH_3-N removed by 3 M NaOH and 4 M H_2SO_4 traps, respectively, was pumped through the incubation flask at a rate of 50 ml min^{-1}. The CO_2 evolved from soil samples was collected in 0.1 M NaOH traps and determined by titration with acid after reacting with saturated $BaCl_2$. The percentage C evolved with time was calculated by subtracting C evolved as CO_2 of the control soil (no organic C amendment) from the various t[illegible] and dividing by the [illegible] residue.

Soil samples were analyzed at 0, 30, 60, and 90 days for 2 M KCl-extractable NH_4^+-N and NO_3^--N by steam distillation (Keeney and Nelson, 1982), TKN (Nelson and Sommers, 1972), Mehlich I extractable Ca, K, Mg, Na, Fe, Zn, and P (Mehlich, 1953), organic C by the Walkley Black method (Nelson and Sommers, 1982), and pH. The ^{15}N analyses were conducted on a Micro Mass 602 spectrometer.

Results and Discussion

Plant Residue Characterization

Characteristics of the fresh plant biomass and anaerobically digested biomass sludge are presented in Table 22. The TC and TKN concentrations of the digested sludges were higher than their respective fresh plant materials. Total C was not significantly different for low and high N fresh plant biomass or digested sludge. The C/N ratio of the fresh plant biomass with a low N content (low N plant biomass) decreased from 35 to 16 after digestion. The C/N ratio of fresh plant biomass with a high N content (high N plant biomass) did not change during digestion.

Lignin content was significantly higher in digested sludges due to loss of readily-decomposable C during anaerobic digestion. The low N fresh plant biomass contained approximately twice as much lignin as the high N fresh plant biomass. Moore and Bjorndal (1984, Unpublished results, Univ. of Florida, Gainesville) concluded that water hyacinth roots, in general, have higher lignin content than shoots. The lignin content of the low N fresh plant biomass was approximately double that of

Table 22 Characteristics of the fresh and digested plant biomass

Chemical	Low N plant biomass		High N plant biomass	
constituent	Fresh	Digested	Fresh	Digested
	---------------- g kg^{-1} of biomass	------------		
Volatile solids	839 b[†]	866 a	837 b	825 c
Ash	181 b	134 c	188 b	173 a
Total carbon	373 b	425 a	385 b	446 a
Lignin	83 b	145 a	43 c	130 a
Cellulose	288 a	180 b	187 b	208 b
Hemicellulose	247 a	243 a	183 b	180 b
Total nitrogen	10.6 c	26.6 b	34.0 a	39.3 a
Calcium	21.0 b	24.2 a	17.8 c	18.4 c
Potassium	22.2 a	2.8 b	23.5 a	3.8 b
Magnesium	8.7 a	2.2 a	3.2 b	2.2 c
Sodium	10.9 c	17.4 b	8.0 d	28.0 a
Iron	1.9 c	7.2 a	1.8 c	4.7 b
Zinc	0.7 c	2.0 a	0.5 d	0.9 b
C/N ratio	35 a	16 b	12 c	12 c

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test

in high N fresh biomass due to a larger rooting mass associated with plants grown in nutrient-depleted water. The low N fresh and digested plant biomass contained more hemicellulose than high N materials. Cellulose content decreased for low N fresh plant biomass after digestion, but increased for high N fresh plant biomass.

Anaerobic digestion resulted in a large loss of K from fresh plant biomass. Potassium is relatively soluble and is used for translocation of anions via the xylem and phloem, enzyme activation, and stomatal movements. Additional K may be required to enhance the microbial degradation of digested biomass sludges when added to soil. The increase in Na concentration in the digested sludges was due to the addition of $NaHCO_3$ buffer to stabilize digester pH. The digestion process resulted in increases in the relative amounts of Ca, Na, Fe, and Zn in the sludge compared to fresh plant biomass.

Carbon and Nitrogen Mineralization

Carbon evolution from the four materials, reported as mg C evolved as CO_2 per g residue C added, is shown in Fig. 16. The soils with fresh plant biomass additions always released more CO_2 compared to that from digested biomass sludges. The two sludges showed similar evolution rates during the first 40 days of incubation despite their difference in TKN. After 40 days, the CO_2 evolution of high N digested sludge increased and continued to increase for the remaining incubation period. This suggests a possible lag period during which the microbial population is adjusting to those species which tolerate high levels of Na. However, there were no significant differences in CO_2 evolution from the two sludges throughout the incubation period (Duncan's multiple range test).

Overall C decomposition cannot be described by simple kinetic equations. However, first-order kinetics describe plant residue or

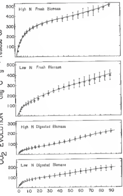

Figure 16 Carbon evolution from soil applied fresh and digested plant biomass.

animal waste decomposition if the overall decomposition sequence is presented as occurring in stages (Gilmour et al., 1977; Hunt, 1977; Reddy et al., 1980). Each stage is thought to represent the sequential ease of C constituent decomposition, i.e. soluble sugars and starch, cellulose and hemicellulose, and lignin.

The rate equation for a first-order reaction is expressed as

$$-dC_i/dt = k_iC_i$$

where subscript i refers to a particular stage of C decomposition. The integrated first-order rate equation is

$$Ct_i = C_i\exp(-k_it)$$

where C_i = C at beginning of a decomposition stage,

Ct_i = C remaining at end of a decomposition stage at time = t,

k_i = first-order rate constant.

Therefore, a rate constant can be calculated for each decomposition stage of an organic C material.

A graphical representation of the stages and their respective rate constants of the materials used in this study are shown in Fig. 17. Decomposition of fresh plant biomass required a three stage first-order kinetic description. Rate constants for the first stage (soluble sugars and starch) were 0.0441 and 0.0222 day^{-1} for high and low N plant biomass, respectively. This stage of decomposition was essentially completed in 4 days. A longer time was required during the second stage (cellulose and hemicellulose) of decomposition for low N plant material due to a higher content of these C constituents (Table 22). The rate constants for the final stage (lignin) of decomposition were low for both materials.

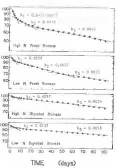

Figure 17 Decomposition stages and rate constants of fresh and digested plant biomass added to soil

Decomposition of digested biomass sludges was adequately described in two stages. The conversion of soluble sugars and starch to CH_4 and CO_2 during anaerobic digestion eliminated the first stage of soluble C degradation for the digested sludges. Therefore, the first stage of sludge decomposition corresponded to the second stage (cellulose and hemicellulose) of fresh plant biomass. The longer second stage corresponded to the final stage (lignin) of fresh plant biomass.

Soil NO_3^--N concentrations during incubation are presented in Table 23. Soil NH_4^+-N concentrations (APPENDIX 8, Table 29) were < 2 mg kg^{-1} during the incubation period. Initial NO_3^--N concentrations were similar for all treatments. No NO_3^--N accumulated in the soil amended with low N fresh plant biomass until 60 days had elapsed. The concentrations of soil NO_3^--N were similar for both digested biomass sludges throughout the incubation despite the higher TKN concentration of the high N sludge. Although the C/N ratios of high N fresh biomass or digested biomass sludge were the same, the amount of NO_3^--N that accumulated in the soil was more than double for fresh biomass compared to digested biomass sludge.

A summary of C and ^{15}N mineralization during the incubation period is presented in Table 24. Approximately 8% of the applied N was recovered as $^{15}NO_3$-N at 90 days for both sludges. Tester et al. (1977) reported 6% of composted sewage sludge N mineralized to NO_3^--N in 54 days. In contrast, 3 and 33% of applied N was recovered as $^{15}NO_3^-$-N for fresh plant biomass with a low and high N content, respectively. Ogwada (1983) incorporated shredded ^{15}N labeled water hyacinth with a C/N ratio of 35 into a Myakka fine sand at a rate of 20 Mg dry wt ha^{-1}. At 60 days, 33% of ^{15}N from the soil-incorporated water hyacinth was

Table 23 Soil NO_3-N concentration from added fresh and digested plant biomass

Day	Control	Low N plant biomass Fresh	Low N plant biomass Digested	High N plant biomass Fresh	High N plant biomass Digested
	----------	----------	mg kg^{-1} soil	----------	----------
0	9.1 a[†]	8.2 ab	7.9 abc	8.4 ab	6.6 c
30	11.0 c	0.9 d	20.0 b	51.4 a	17.2 b
60	13.2 c	1.0 d	24.4 b	70.3 a	24.8 b
90	14.5 c	4.8 d	28.4 b	68.8 a	30.3 b

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test.

Table 24. Carbon and ^{15}N mineralization from added fresh and digested plant biomass.

	Low N plant biomass		High N plant biomass	
	Fresh	Digested	Fresh	Digested
	------------% of added C or N			--------
		Day 30		
C	22.4 b[†]	11.2 c	34.2 a	9.8c
^{15}N	ND[‡]	4.7 b	24.8 a	3.8 b
		Day 60		
C	32.5 b	14.9 c	41.8 a	16.1 c
^{15}N	ND	8.2 b	34.3 a	6.0 b
		Day 90		
C	38.0 b	19.1 c	49.9 a	23.1 c
^{15}N	3.3 c	7.7 b	33.3 a	7.7 b

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test.

[‡] ND = not detectable.

mineralized and 20% of the initial ^{15}N was recovered in sorghum-sudangrass plants.

After 90 days of incubation, approximately 20% of the added C of digested biomass sludges had evolved as CO_2 compared to 39 and 50% of fresh plant biomass with a low and high N content, respectively. More than half of the C evolved from fresh plant biomass occurred within the first 10 days.

The percentage of C evolved with time from anaerobically digested biomass sludge is similar to results of Miller (1974) and Tester et al. (1977) for anaerobically digested and composted sewage sludge. Miller (1974) reported a maximum of 20% of the added C was evolved as CO_2 during a 6-month incubation period. Tester et al. (1977) reported that 16% of the added C was evolved as CO_2 from composted sewage sludge during 54 days of incubation. However, Terry et al. (1979) found a total of 26 to 42% of C was evolved as CO_2 from anaerobically digested sewage sludge during 130 days of incubation.

Carbon/nitrogen ratio is commonly used as a guideline for predicting the relative decomposability or mineralization potential of organic materials added to soil (Reddy et al., 1980). The high C/N ratio of low N fresh plant biomass (C/N = 35) resulted in immobilization of inorganic N. The low C/N ratio of high N fresh plant biomass (C/N = 12) resulted in rapid mineralization of organic N. However, C/N ratios may have limited applicability for predicting N transformations of digested biomass sludges. Both high N fresh biomass and digested biomass sludge had C/N ratios of 12, but only 8% of the applied N was recovered as $^{15}NO_3^-$-N from digested sludge compared to 33% from fresh plant biomass.

Other Soil Parameters

Soil pH increased by one and two pH units after addition of anaerobically digested biomass sludge with a low and high N content, respectively (Table 25). The addition of fresh plant biomass did not appreciately alter the initial soil pH. During the 90 day incubation, soil pH generally decreased with all residue additions, except for low N fresh plant biomass.

The Na content of the digested biomass sludges explains, in part, the increase in the initial soil pH. Atalay and Blanchar (1984) found that addition of anaerobically digested plant residue to soil increased pH from 5.5 to 7.6. They attributed the pH increase to a limestone buffer used during anaerobic digestion.

The addition of fresh or anaerobically-digested water hyacinth increased Mehlich 1 extractable soil constituents after 90 days of incubation (Table 26). Mehlich 1 extractable soil constituents at 0, 30 and 60 days of incubation are presented in APPENDIX E, Tables 30 to 32. The increases were a direct reflection of the mineral composition of the respective residue (Table 22). There was a large increase in soil K with fresh plant biomass additions compared to digested biomass sludges. All treatments resulted in large increases of soil Na and Ca. Parra and Hortenstine (1976) concluded that fresh water hyacinths contained appreciable amounts of soluble salts and that crops susceptible to salt injury should not be planted immediately after soil additions.

[illegible] from added [illegible] and digested plant biomass.

Day	Control	Low N plant biomass		High N plant biomass	
		Fresh	Digested	Fresh	Digested
0	5.44 d[†]	5.38 d	6.49 b	5.73 c	7.34 a
30	5.48 c	5.99 b	6.27 a	4.80 d	6.21 a
60	4.99 c	5.86 ab	6.03 a	4.30 d	5.77 b
90	5.42 b	5.94 a	6.03 a	4.72 c	6.02 a

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test.

Table 20. [illegible] extractable [illegible] and digeste [illegible]

Chemical constituent	Control	Low N plant biomass Fresh	Low N plant biomass Digested	High N plant biomass Fresh	High N plant biomass Digested
		-mg kg^{-1} soil-			
Calcium	252 e[†]	361 ab	373 a	327 d	348 bc
Potassium	25 e	115 b	40 d	119 a	47 c
Magnesium	24 d	59 a	35 c	39 b	35 c
Sodium	3 e	53 c	72 b	41 d	117 a
Iron	14 d	14 d	20 a	15 c	18 b
Zinc	3 3 e	5 7 d	11 3 a	6 0 c	7 2 b

[†] Values with same letter within rows are not significantly different at 0 05 level by Duncan's Multiple Range Test

Conclusions

After 90 days of incubation, approximately 20% of the added C of the digested biomass sludges had evolved as CO_2 compared to 39 and 50% of the fresh plant biomass with a low and high N content, respectively Decomposition of fresh plant biomass followed a three stage first-order kinetic description Decomposition of digested sludge was adequately described by two stage first-order kinetics

Mineralization of organic N to $^{15}NO_3^-$-N accounted for approximately 8% of applied N for both digested biomass sludges at the end of 90 days Nitrogen mineralisation accounted for 3 and 33% of applied N for fresh plant biomass with a low and high N content, respectively

The soil pH increased after addition of digested biomass sludge, but was not appreciately altered after addition of fresh plant biomass The Na content of digested sludges was attributed as the primary factor for increasing soil pH

Increases in Mehlich I extractable soil constituents were a direct reflection of the mineral composition of fresh or digested plant biomass The high Na concentration of digested biomass sludge suggests pretreating the sludge to remove some of the salts and selecting salt tolerant plants if the sludge is used as a soil amendment

MASS BALANCE OF NITROGEN IN AN INTEGRATED "BIOMASS FOR ENERGY SYSTEM

A series of experiments were conducted to evaluate N cycling in three components of an integrated "biomass for energy" system, i.e. water hyacinth production, anaerobic digestion of hyacinth biomass, and recycling of digester effluent and sludge. Two types of water hyacinth biomass production systems were evaluated: i) plants growing in nutrient-enriched waters representing wastewater treatment systems (sewage and digester effluents, animal wastes or agricultural drainage water), and plants growing in nutrient limited systems representing natural waters receiving low external N inputs.

Nutrient-Enriched Systems

Nitrogen cycling in an integrated system for water hyacinths growing in nutrient-enriched systems is summarized in Fig. 18.

Water Hyacinth Production

A wide range of productivity (5 to 64 g dry wt m^{-2} day^{-1}) has been recorded for plants growing in nutrient-enriched waters (Boyd, 1976, Hanisak et al., 1980; Reddy and DeBusk, 1983; Reddy et al., 1985). Plants growing in nutrient-enriched media generally have an N content of 30 to 40 g N kg^{-1} of dry tissue (Boyd, 1976; Wolverton and McDonald, 1979, Reddy et al., 1985). In this study the tissue N content of plants growing in sewage effluent was 35 g N kg^{-1} of dry biomass.

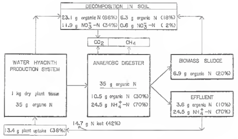

Figure 18. Nitrogen cycling in an integrated system for water hyacinths growing in nutrient-enriched systems. Numbers in parentheses are percentages of the initial 35 g N placed in the anaerobic digester

Although plant assimilation is a major process for N removal in ponds of water hyacinths, other N transformations may occur in the root zone, water column and underlying sediment. These processes account for much of the loss of N from the system. For example, a dense mat of water hyacinth could potentially enhance denitrification of NO_3^--N present in the water column (Boyd, 1970, Reddy, 1981). Total N reduction in water hyacinth systems was reported in a range from 65 to 94% of various wastewaters (Sheffield, 1967, Clock, 1968, Hanisak et al., 1980, Reddy et al., 1982).

Total fertilizer N recovered by plants in this study ranged from 50% in a field study to 90% in controlled-greenhouse studies. Results indicated that detritus accounted from 3 to 14% of the total biomass produced in water hyacinth ponds. Up to 28% of the total plant N was found in detritus, which could potentially be deposited at the sediment-water interface.

Anaerobic Digestion of Hyacinth Biomass

Anaerobic digestion of biomass with high tissue N and low C/N ratio resulted in mineralization of 70% of plant organic N to NH_4^+-N. Total N recovered for digested high N plants was ~100% of the added N which agreed with results by Hashimoto et al. (1980) and Field et al. (1984). After digestion, 80% of the plant organic N was recovered in the effluent and 20% was recovered in the sludge.

Waste By-Product Recycling

Undiluted and diluted effluents were used as nutrient sources to produce additional water hyacinth biomass and for potential N recovery. Plants growing in undiluted effluents did not survive the combination of high salt and NH_4^+ N concentrations. However, diluting the digester effluents provided a media which stimulated water hyacinth growth

Plant assimilation of effluent N [illegible] of the initial N placed in the digester. Nitrogen loss from this component of the integrated system accounted for 42% of the N placed in the digester. Possible N loss mechanisms during recycling of digester effluent include algal assimilation and NH_3-N volatilization.

The digested sludge contained 20% of the initial N placed in the digester. The sludge N could potentially be recovered during crop production after land application. The sludge was fairly resistant to decomposition in soil and only 20% of the sludge C was evolved as CO_2 in 90 days of incubation. A low decomposition rate of sludge applied to soil was attributed to loss of the more readily-decomposable C constituents during anaerobic digestion. The decomposition rates observed in this study for digested biomass sludge applied to soil were found to be similar to those reported for digested sewage sludge (Miller, 1974; Tester et al., 1977). Nitrogen mineralization of sludge organic N during 90 days of decomposition in soil accounted for only 2% of the initial N placed in the digester.

Fresh water hyacinth biomass was also added to soil to compare its decomposition to that of digested sludge. Approximately 50% of the biomass C was evolved as CO_2 during 90 days of incubation. Nitrogen mineralization of biomass organic N accounted for 34% of the initial plant N placed in the soil. Mixing the fresh water hyacinth biomass with anaerobically digested sludge may enhance the decomposition of the sludge.

Total N recovery by sludge and effluent recycling in the integrated 'biomass for energy' system was 60% of the initial N placed in the digester for high N plants. The remaining 40% was lost from the system during effluent recycling in a water hyacinth production system.

Nutrient-Limited Systems

Nitrogen cycling in an integrated system for water hyacinths growing in nutrient-limited systems is summarized in Fig. 19.

Water Hyacinth Production

Growth rates of 2 to 20 g dry wt m^{-2} day^{-1} have been reported for plants in natural waters of central and south Florida (Yount and Crossman, 1970; DeBusk et al., 1981). Plants growing in nutrient-limited systems generally have a low N content. In this study, tissue N content of plants grown in tap water without nutrients was 10 g N kg^{-1} of dry biomass. In addition to a low N content, the shoot:root dry weight ratio decreased as nutrient availability decreased in the water media (Reddy, 1984). The shoot root dry weight ratio of plants with a low N content was 1.6 compared to 4.2 of plants with a high N content.

Anaerobic Digestion of Plant Biomass

Shiralipour and Smith (1984) concluded that increasing N in the growth medium used for water hyacinth production increased methane yields during anaerobic digestion. Surprisingly, gas yields were higher for plants with a low N content compared to plants with a high N content. However, the gas yield differences were attributed to characteristics of inoculum used during digestion. Only 75% of the initial N placed in the digester was recovered in the effluent and sludge for low N plants. The low total N recovery is difficult to explain. Only 35% of the plant organic N placed in the digester was mineralized to NH_4^+-N compared to 70% mineralization for high N plants. Approximately 40% of the initial N placed in the digester remained as organic N after digestion. After digestion, 54% of the initial plant organic N was recovered in the effluent while 20% was recovered in the digested sludge.

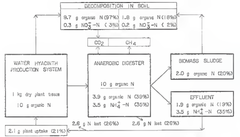

Figure 19. Nitrogen cycling in an integrated system for water hyacinths growing in nutrient-limited systems. Numbers in parentheses are percentages of the initial 10 g N placed in the anaerobic digester.

Waste By-Product Recycling

The undiluted effluent from digested plants with a low N content was recycled directly to a water hyacinth production system. Plants survived in undiluted effluents due to lower NH_4^+-N concentrations. Approximately 21% of the N placed in the digester was reassimilated by water hyacinths during effluent recycling. Nitrogen loss during digestion and effluent recycling accounted for 52% of the initial N placed in the digester.

The digested sludge contained 20% of the initial N placed in the digester. Similar decomposition rates were noted for both digested sludges applied to soil despite their differences of initial N content (27 and 39 g N kg^{-1} dry sludge). The mineralization of sludge organic N accounted for 2% of the initial N placed in the digester.

The fresh water hyacinth biomass decomposed rapidly in soil but only 3% of the applied N was mineralized to NO_3-N. Total N recovery by sludge and effluent recycling was 46% of the initial N placed in the digester for low N plants. The remaining N was lost from the system during anaerobic digestion and effluent recycling.

CONCLUSIONS

The three components of the integrated 'biomass for energy'' system were 1) the water hyacinth biomass production system, 2) anaerobic digestion of water hyacinth biomass, and 3) waste recycling of digested biomass sludge and effluent Nitrogen cycling was investigated for each component of the integrated system

Water Hyacinth Productivity and Detritus Production

Productivity of water hyacinth was influenced by ambient air temperature, solar radiation, and nutrient composition of the medium The highest net productivity occurred during spring and summer and over 75% of the biomass produced was recorded during this time period The detritus production exceeded net biomass production during winter regardless of water fertility.

Seasonal yields of water hyacinth ranged from 1 9 to 23 1 Mg (dry wt) ha^{-1} and 0 2 to 10 2 Mg ha^{-1} for plants growing in eutrophic lake water with and without added nutrient, respectively Detritus comprised 3 to 14% of the total biomass and detritus production was not significant between reservoirs or seasons. Although detritus production was similar for both reservoirs, fertilization resulted in significant increases in detritus N content Nitrogen loading to the reservoirs from detritus was 148 and 92 kg N ha^{-1} yr^{-1} for plants grown in eutrophic lake water with and without added nutrients, respectively.

Approximately 30% of the fertilizer N was recovered by plants. Total N assimilated by water hyacinth (live plants and detritus) was 720 and 325 kg N ha^{-1} yr^{-1} for plants grown in eutrophic lake water with and without added nutrients, respectively.

Detritus and Nitrogen Transformations

Detritus had no apparent effect on rate of N loss in water with water hyacinth plant cover due to rapid plant assimilation. However, N loss in water without plant cover was more rapid with detritus additions. Both sediment and detritus appeared to be potential N sources for plant assimilation.

Total N recovered by water hyacinth ranged from 57 to 72% for added $^{15}NO_3^-$-N and 70 to 89% for added $^{15}NH_4^+$-N. Less than 10% of added $^{15}NH_4^+$ N was immobilized by detritus in water with plant cover. However, up to 35% of the added $^{15}NH_4^+$ N was associated with detritus in water without plant cover. This suggests that during periods of low water hyacinth productivity, i.e. winter, detritus is an important sink for added N.

Increasing amounts of detritus generally decreased dissolved O_2 concentrations of water. The pH of water without plant cover generally was lower when detritus was added.

Anaerobic Digestion of Water Hyacinth

Initial water hyacinth N content and volume of inoculum did not affect long term (60 days) biogas production. At 15 days, biogas production was generally greater for plants with a high N content. Inoculum volume showed little effect of biogas production for low N plants throughout incubation. However, a larger volume of inoculum increased biogas production for plants with a high N content after [illegible] days of digestion.

Removing the biomass sludge from digester effluents decreased the electrical conductivity, NH_4^+-N and TKN of the effluent. Anaerobic digestion resulted in a loss of K and Mg from fresh plant biomass but increased lignin, total C, TKN, Ca, Na, Fe and Zn of the digested sludge. Generally cellulose decreased during digestion and hemicellulose remained unchanged.

Mineralization of organic N to NH_4^+-N was the primary N transformation occurring during anaerobic digestion. Approximately 20% of the organic N placed in the digesters was recovered as sludge organic N. Net mineralization of organic N to NH_4^+-N was 70 and 35% of the added organic N of plants with a high and low N content, respectively.

Digester Effluent Recyling

The initial electrical conductivities and NH_4^+-N concentrations of the digester effluents ranged from 0.7 to 6.7 dS m^{-1} and 23 to 289 mg N L^{-1}. The highest water hyacinth dry weight gains were associated with the lowest electrical conductivities. However, plants survived in effluents having electrical conductivities of 5.6 and 5.9 dS m^{-1}. Plant death was observed in four undiluted effluents. Additional information would be needed to establish optimum dilution of anaerobic digester effluents to promote maximum biomass yields.

First-order kinetic equations were used to describe NH_4^+-N loss from effluent with k values ranging from 0.175 to 0.593 day^{-1}. Generally the highest k values were associated with rapid plant growth. Plant ^{15}N assimilation was observed for all effluents although plant death resulted in low recoveries ([illegible] to 16%). The ^{15}N recovery for other effluents ranged from 36 to 77%. The majority of the ^{15} was recovered in shoot material.

Plants grown in diluted effluents assimilated large amounts of Na and K and net shoot assimilation of all nutrients was greater than net root assimilation. Dead plants showed a net loss of K but net gains of other nutrients.

Digested Sludge Recycling

Decomposition of fresh and anaerobically digested water hyacinth biomass added to soil was evaluated by CO_2 evolution and ^{15}N mineralization. Approximately 39 and 50% of the added C was evolved as CO_2 in 90 days for fresh plant biomass with a low and high tissue N content, respectively. Approximately 20% of the added C was evolved as CO_2 for both digested sludges in 90 days. Decomposition of fresh plant biomass required a three stage first-order kinetic description of C loss compared to a two stage first order description for digested sludge. Apparently the loss of readily-decomposable C during anaerobic digestion eliminated the first stage of decomposition in soil.

Only 8% of the applied ^{15}N was mineralized to NO_3^--N for digested sludges despite their differences of initial N content. In contrast, 3 and 33% of the applied ^{15}N was recovered as NO_3^--N for fresh biomass with a low and high N content, respectively. The C/N ratio of digested sludge was not a predictive guide for N mineralization potential. Mixing the fresh biomass with digested sludge may enhance the decomposition of the sludge in soil.

APPENDIX A

DIGESTER EFFLUENT CHARACTERISTICS DURING WATER HYACINTH TREATMENT

Table 27. Effluent pH during water hyacinth treatment

Inoculum volume	Day 0	2	4	6	8	10	12	14	16	18	20	22
--L--	pH											
	Diluted effluents from high N plants											
2.5	7.8	7.7	7.7	7.6	7.5	7.3	7.5	7.2	7.4	7.3	7.1	7.6
5	7.7	7.7	7.9	7.8	7.8	7.8	7.6	7.4	7.6	7.8	7.1	7.9
10	7.7	8.0	8.1	7.9	7.9	7.6	7.7	7.4	7.8	7.8	7.1	7.5
	Undiluted effluents from high N plants											
2.5	7.6	8.1	8.3	8.4	8.7	8.4	8.0	8.0	8.3	8.2	8.3	8.1
5	7.6	8.0	8.2	8.2	8.3	8.6	8.7	8.3	8.8	8.8	9.0	8.8
10	7.6	8.0	8.2	8.2	8.2	8.4	8.3	8.0	8.3	8.5	8.8	8.8
	Undiluted effluents from low N plants											
2.5	7.5	8.1	8.3	8.4	8.5	NA[†]	NA	8.8	8.8	8.7	8.8	8.8
5	7.4	8.2	8.3	8.5	8.6	NA	NA	9.2	9.1	9.2	9.2	9.1
10	7.5	8.1	8.3	8.5	8.6	NA	NA	9.3	9.3	NA	NA	NA
	Nutrient medium											
	7.5	6.4	4.8	3.9	3.6	NA	NA	3.3	3.5	3.4	3.5	3.5

[†] NA = Not available

Table 28 Effluent dissolved O_2 concentration during water hyacinth treatment

Inoculum volume	Day 0	2	4	6	8	10	12	14	16	18	20	[illegible]
—L—						mg O_2 L^{-1}						
						Diluted effluents from high N plants						
2.5	6.2	4.6	5.4	5.2	4.9	4.7	5.3	5.6	3.9	3.8	3.7	3.3
5	5.4	4.4	3.2	4.0	4.0	4.1	4.5	4.6	3.2	3.5	3.4	2.8
10	4.4	3.5	3.6	2.8	3.5	1.8	4.6	4.3	3.0	3.2	3.7	3.3
						Undiluted effluents from high N plants						
2.5	1.8	2.6	2.9	0.6	0.5	1.0	2.6	2.3	2.9	2.9	3.9	3.1
5	1.7	2.9	2.1	1.0	0.4	0.8	2.9	2.5	2.8	2.5	3.8	2.1
10	0.4	2.1	1.4	0.7	0.2	0.3	2.2	1.3	1.1	1.3	1.5	1.0
						Undiluted effluents from low N plants						
2.5	4.1	4.5	3.1	2.7	5.1	NA[†]	NA	8.0	7.0	8.0	5.9	7.2
5	4.1	4.7	3.7	2.1	3.8	NA	NA	11.9	12.1	11.4	6.9	9.1
10	3.9	4.8	3.5	1.1	1.4	NA	NA	9.1	11.0	NA	NA	NA
						Nutrient medium						
	7.1	6.9	6.3	5.7	7.0	NA	NA	6.7	7.0	7.2	6.3	[illegible]

[†] NA = Not available.

APPENDIX B

SOIL CHARACTERISTICS FROM ADDED FRESH AND ANAEROBICALLY DIGESTED PLANT BIOMASS

Table 29. Soil ammonium concentrations following fresh and digested plant biomass.

Day	Control	Low N plant biomass		High N plant biomass	
		Fresh	Digested	Fresh	Digested
	-----------	----g kg^{-1}	-	-----------------	-----
0	1.2 b[†]	2.2 b	1.7 b	25.1 a	1.9 b
30	1.2 a	1.4 a	1.8 a	1.8 a	1.3 a
60	1.1 a	1.2 a	1.7 a	1.0 a	1.0 a
90	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test

Table 30. Mehlich I extractable constituents at day 0 from added fresh and digested plant biomass

Chemical constituent	Control	Low N plant biomass		High N plant biomass	
		Fresh	Digested	Fresh	Digested
	------------		--mg kg^{-1}	-------	
Calcium	257 c[†]	377 a	344 ab	332 b	351 ab
Potassium	27 d	128 a	44 c	127 a	53 b
Magnesium	25 d	55 a	34 c	37 b	33 c
Sodium	7 e	60 c	70 b	56 d	133 a
Iron	18 d	25 b	35 a	21 c	26 b
Zinc	4.1 d	7.3 b	13.3 a	6.1 c	7.2 b

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test

Table 31 Mehlich I extractable constituents at day 30 from [illegible] fresh and digested plant biomass

Chemical		Low N plant biomass		High N plant biomass	
constituent	Control	Fresh	Digested	Fresh	Digested
			mg kg^{-1}		
Calcium	245 c[†]	396 a	383 a	340 b	379 a
Potassium	39 c	139 a	55 c	124 b	49 c
Magnesium	26 c	68 a	39 b	39 b	34 bc
Sodium	3 e	57 c	75 b	41 d	114 a
Iron	17 c	18 b	25 a	15 d	18 b
Zinc	4.1 d	6.1 c	11.9 a	6.1 c	7.7 b

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test.

Table 32 Mehlich 1 extractable constituents at day 60 from added fresh and digested plant biomass

Chemical		Low N plant biomass		High N plant biomass	
constituent	Control	Fresh	Digested	Fresh	Digested
	mg kg^{-1}				
Calcium	276 c[†]	359 b	393 a	343 b	357 b
Potassium	27 d	113 b	44 c	123 a	48 c
Magnesium	27 d	56 a	39 b	40 b	34 c
Sodium	4 e	55 c	74 b	38 d	119 a
Iron	16 c	16 c	23 a	16 c	18 b
Zinc	4.5 d	6.5 bc	12.8 a	6.0 c	7.3 b

[†] Values with same letter within rows are not significantly different at 0.05 level by Duncan's Multiple Range Test

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BIOGRAPHICAL SKETCH

Kevin Moorhead was born in Findlay, Ohio, on May 22, 1956, and spent the first 18 years of his life there. After high school graduation and a summer in California, Kevin moved to Swannanoa, North Carolina, and began his college career at Warren Wilson College. He graduated in December, 1978, with a Bachelor of Arts degree in biology. Kevin moved to Prattville, Alabama, and worked for Alaga-Whitfield food company in Montgomery, Alabama, for 7 months. In August, 1979, he moved to Columbus, Ohio, to begin a graduate program in agronomy at the Ohio State University. Kevin received his Master of Science degree in December, 1981, with an emphasis in soil fertility, under the direction of the late Dr. E. O. McLean. Since January, 1982, he has pursued a Ph.D. degree at the University of Florida in the Department of Soil Science. He is presently a candidate for the degree of Doctor of Philosophy with an emphasis in soil biochemistry. Kevin has 7 sisters, a brother looking for Sasquatch, and (at this point) 3 nieces and 1 nephew.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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